

## Proconvulsant effects of tramadol and morphine on pentylenetetrazol-induced seizures in adult rats using different routes of administration

Morteza Gholami <sup>a</sup>, Ehsan Saboory <sup>b,\*</sup>, Shiva Roshan-Milani <sup>c</sup>

<sup>a</sup> Department of Biology, Faculty of Sciences, University of Urmia, Iran

<sup>b</sup> Neurophysiology Research Center, Urmia University of Medical Sciences, Iran

<sup>c</sup> Department of Physiology, Faculty of Medicine, Urmia University of Medical Sciences, Iran

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### ABSTRACT

Tramadol is frequently used as a pain reliever. However, it has been sometimes noted to have the potential to cause seizures. Because of its dual mechanism of action (both opioid and nonopioid), the adverse effect profile of tramadol can be different in comparison with single-mechanism opioid analgesics, such as morphine. In the present study, the facilitatory effects of tramadol and morphine on pentylenetetrazol-induced seizures using different routes of administration were compared in rats. Adult female rats were divided into six groups and continuously received saline, morphine, or tramadol on a daily basis for 15 days [gavage (PO) or intraperitoneal (IP)]. An increasing dose of morphine and tramadol was used to prevent resistance to repetitive dose (20–125 mg/kg). Following one week of withdrawal period and 30 min before the seizure induction (PTZ = 80 mg/kg, IP), each group of rats was further divided into subgroups that received saline, morphine, or tramadol for the second time on the 22nd day of the experiment. Results showed that, while morphine, tramadol, and their administration had different effects on seizure behaviors, both acute and chronic administrations of morphine and tramadol potentiated PTZ-induced seizures. However, there was no significant difference between morphine and tramadol in terms of seizure severity. Effects of morphine and tramadol on PTZ-induced seizures were also stable following one week of withdrawal. In conclusion, this study indicated similar severity in the proconvulsant effect of morphine and tramadol on PTZ-induced seizures, which might depend on their similar effects on GABAergic pathways.

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### 1. Introduction

Epilepsy is characterized by recurrent, unprovoked seizures with any immediately identifiable cause [1–3]. It is a common neurological disorder that affects individuals of all ages [2]. In recent years, some animal models of epilepsy have been developed, which include genetic animal models, chemical-induced epilepsy models, and kindling models [4,5]. These models have played a fundamental role in testing novel antiepileptic drugs (AEDs) and helped in determining the pathological and physiological pathways associated with human epilepsy [5]. Pentylenetetrazol (PTZ) is a convulsant chemical agent that has been frequently used in experimental models for seizure induction [6,7]. This noncompetitive antagonist blocks GABA-mediated Cl<sup>-</sup> influx through an allosteric interaction in the Cl<sup>-</sup> channel, thus leading to

the depolarization of neuronal membrane and propagation and maintenance of seizure activities. Acute effects of PTZ are largely mediated by its action in the GABAA receptors [8–10]. Nevertheless, some contributions by other receptors have also been made. Discriminative stimulus effects of PTZ can be modulated by nicotinic [11,12] and NMDA [13] receptors and brain monoaminergic systems such as serotonin and dopamine [14–17]. Previous reports have indicated the critical role of brain monoamine in establishing the convulsion threshold [18]. It has also been shown that exposure to PTZ increases the density of metabotropic glutamate receptor and activity of the opioid system and that it causes a number of biologic alterations in the hippocampus including inositol triphosphate formation in PTZ-kindled rats [19,20].

Morphine use and abuse can also alter seizure threshold [21,22]. However, the activity of the opiate system influences the expression of seizures in contrasting ways, depending on opiate dose and mode of seizure induction [23]. Morphine can modulate seizure susceptibility in a biphasic manner [24,25] and cause dose-dependent anticonvulsant and proconvulsant effects. Intracranial administration of morphine and opioid peptides elicits pathologic epileptiform activity in the

\* Corresponding author at: Department of Physiology, Faculty of Medicine, Urmia University of Medical Sciences, Iran. Tel.: +98 4412770698; fax: +98 4412780801.

E-mail addresses: [biologygholami@gmail.com](mailto:biologygholami@gmail.com) (M. Gholami), [saboory@umsu.ac.ir](mailto:saboory@umsu.ac.ir), [e.saboory@yahoo.com](mailto:e.saboory@yahoo.com) (E. Saboory), [shiva\\_muk@yahoo.com](mailto:shiva_muk@yahoo.com) (S. Roshan-Milani).

electroencephalogram [26] and may induce seizures in humans [27]. A potential withdrawal condition caused by the abrupt discontinuation of opiate intake after an extended period of abuse may also induce seizure-like activity [28].

Tramadol hydrochloride, which is widely used throughout the world, is a centrally acting analgesic prescribed for moderate-to-severe pain [29,30]. Tramadol and morphine bind to  $\mu$ -opioid receptors; however, tramadol has several-time weaker affinity with this receptor than morphine [30]. Tramadol does not precipitate withdrawal symptoms [31]. Several reports and controlled laboratory studies have indicated that it may be effective in relieving opioid withdrawal symptoms [32,33]. Despite its affinity with the opiate receptor, tramadol is not chemically related to opiates [34]. However, its effects are attributed to its opioid and nonopioid (inhibition of noradrenaline and serotonin reuptake) actions [35,36]. In preclinical evaluation, tramadol displays both proconvulsant and anticonvulsant properties [37–39]. Some studies have indicated that tramadol can only provoke seizures if used in excessive doses in patients with epilepsy or if coadministered with other seizure-inducing drugs [40,41]. Because of tramadol's dual mechanism of action, its adverse effect profile can be different in comparison with single-mechanism opioid analgesics, such as morphine. Despite numerous studies of the dose-dependent and biphasic effects of tramadol and morphine on seizures, there is still lack of knowledge about the comparison of specific effects of morphine and tramadol on seizures, especially PTZ-induced seizures, following acute and chronic administration or withdrawal using different methods of administration (IP or PO). On the other hand, because multiple receptor systems are involved in triggering opioid-induced seizures, such as opioid, adrenergic, glutamatergic [42], and opioid antagonism of inhibitory GABA neurotransmission [43], it seems that there are some interactions between PTZ- and opioid-induced seizures. Therefore, in the present study, PTZ-induced seizure was used as a model in order to determine whether administration (or withdrawal) of morphine and tramadol would augment PTZ-induced seizures in rats or not.

## 2. Materials and methods

### 2.1. Animals

Adult female Wistar rats ( $n = 72$ ) weighing 180–200 g were housed in Plexiglas cages in a colony and maintained at  $22 \pm 2$  °C with a 12-h light/dark cycle (lights were turned on at 0700). These rats were allowed free access to food and water. Bedding consisted of untreated wood shavings and was changed three times a week. All the experimental protocols and procedures were in agreement with the guidelines of the 1975 Declaration of Helsinki, as reflected in the guidelines of Medical Ethics Committee, Ministry of Health, Iran. In addition, Regional Medical Ethics Committee of West Azerbaijan Province, Islamic Republic of Iran, approved this study.

### 2.2. Drug administration

The rats were randomly divided into three groups for either intraperitoneal (IP) or gavage (PO) administrations by saline, morphine (Temad Co., Tehran, Iran), and tramadol (Atlantis Life Sciences Co., Mumbai, India). Rats in morphine and tramadol groups received morphine or tramadol with an increasing dose (20, 27.5, 35, 37.5, 45, ..., until 125 mg/kg) once per day for 15 consecutive days. Administered dose of morphine/tramadol was increased by 7.5 mg/kg every day. These increasing doses of tramadol (IP) have been previously used in different studies [44]. The saline group received saline in a similar manner. Following one week of withdrawal period, the rats in all the groups were further divided into subgroups on the 22nd day of the experiment. In the saline (IP and PO) groups, a total number of 30 rats were divided into 3 subgroups which received saline [saline/saline = SS: SS IP ( $n = 5$ ) and SS PO ( $n = 5$ )], morphine

[saline/morphine = SM: SM IP ( $n = 5$ ) and SM PO ( $n = 5$ )], or tramadol [saline/tramadol = ST: ST IP ( $n = 5$ ) and ST PO ( $n = 5$ )]. In tramadol groups, a total number of 22 rats were divided into 2 subgroups which received saline [tramadol/saline = TS: TS IP ( $n = 5$ ) and TS PO ( $n = 5$ )] or tramadol [tramadol/tramadol = TT: TT IP ( $n = 7$ ) and TT PO ( $n = 5$ )]. Finally, in morphine groups, 20 rats were divided into 2 subgroups which received saline [morphine/saline = MS: MS IP ( $n = 5$ ) and MS PO ( $n = 5$ )] or morphine [morphine/morphine = MM: MM IP ( $n = 5$ ) and MM PO ( $n = 5$ )]. Rats in SM and ST groups were considered to have acute drug administration because they were not exposed to morphine or tramadol during 15 days of the treatment. Similarly, those in MS and TS groups were considered to have chronic drug administration because of continuously receiving an increasing dose of morphine or tramadol for 15 days to establish the dependence model. Rats in MM and TT groups, with one week of withdrawal period, were considered to present a model of chronic use, withdrawal, and relapse. Rats of all the groups received PTZ (80 mg/kg, IP) 30 min after saline, morphine, or tramadol administration. All morphine, tramadol, and saline administrations occurred at the same time each day (1100 and 1200). Morphine was dissolved in 0.9% saline and freshly prepared for each use; however, tramadol hydrochloride was in the liquid form. Effects of drug administration on PTZ-induced seizure behaviors were investigated one week later because the previous study of the present authors showed that the increasing doses of morphine and tramadol in a neonatal period had a long-term effect (a week later) on PTZ-induced seizures [45]. To avoid different estrous cycles in rats and minimize effect of sex hormone fluctuations on their behavior [46], all the subjected rats were arranged to be in metestrous period when the experiment was started [47]. The rats were gently held in the hand, and a vaginal smear was obtained in the morning (9 h). Sterile cotton-tipped swabs wetted in distilled water were gently introduced into the vaginal orifice; the introduction was relatively shallow (approximately 1 cm) to avoid excessive cervical stimulation and a consequent pseudopregnancy. Subsequently, they were carefully rotated (one twist) against the vaginal wall [48]. Rats were not anesthetized during smear collection. The vaginal smear was mounted on a glass slide with a cover slip and was observed under light microscopy. The metestrous period was identified with high number of leukocytes as well as few nucleated epithelial cells [48,49]. If a rat was not in the metestrous period, she was returned to her home cage and was retested 1–3 days later according to the result of her vaginal smear. However, it is likely that the females may not have been selected exactly at the same cycle stage because the method used here was not very accurate in distinguishing the cycle stage. We avoided collecting vaginal smears of the females for four consecutive days to minimize handling-induced stress in subjects.

### 2.3. Apparatus

The seizure test cage was a round, plywood cage 81.5 cm in diameter, closed by a wall which was 31.5 cm high. The light source was a 150-W lamp, hanging 1.20 m above the floor level, similar to the one used by Martin-Garcia and Pallares [50].

### 2.4. Seizure testing

Rats were transferred to the testing room one day before the experiment to be acclimatized to the new environment. A seizure was induced by the IP injection of PTZ (80 mg/kg) [51,52] on the 22nd day of the experiment. Immediately after the injection, the rats were individually placed in the center of the apparatus, and their behaviors were videotaped and monitored for 40 min. They were then tested in a random order. The seizure test cage was cleaned at the end of each trial to prevent behavioral modifications due to the presence of odor. All the rats were seizure-naive when tested, and each one was subjected

to seizure testing only once. Seizures were induced between 13 and 16 h to minimize the possible confounding effects of circadian rhythms [53]. These seizures were assessed using a previously defined scale [14] in which 0 = no response, 1 = ear and facial twitching, 2 = myoclonic jerks without rearing, 3 = myoclonic jerks with rearing, 4 = turning over onto one side with tonic-clonic seizures, and 5 = turning onto back with generalized tonic-clonic convulsions. The monitored parameters were as follows: severity of seizure, time to onset of the first seizure behavior (time to onset of level 1 behavior: ear and facial twitching, s), duration of myoclonic jerks and tonic-clonic seizures (s), duration of level 4 behavior (s), duration of immobility (min), number of myoclonic jerks, percentage of level 4 behavior, percentage of mortality, and latency of myoclonic jerks (min).

### 2.5. Statistical analysis

Statistical analysis was performed using SPSS 16.0 software. Data were expressed as mean  $\pm$  SEM for each experimental group. Two-group comparisons were performed using *t*-test, whereas multiple-group comparisons were performed using one-way analysis of variance (ANOVA). When appropriate, Tukey's test was used for post hoc analyses. The data related to mortality rate and percentage of level 4 behavior were analyzed using Fisher's exact test [54,55]. Results were considered significant at  $p < 0.05$ .

## 3. Results

### 3.1. Effects of morphine and tramadol on time to onset of PTZ-induced seizures

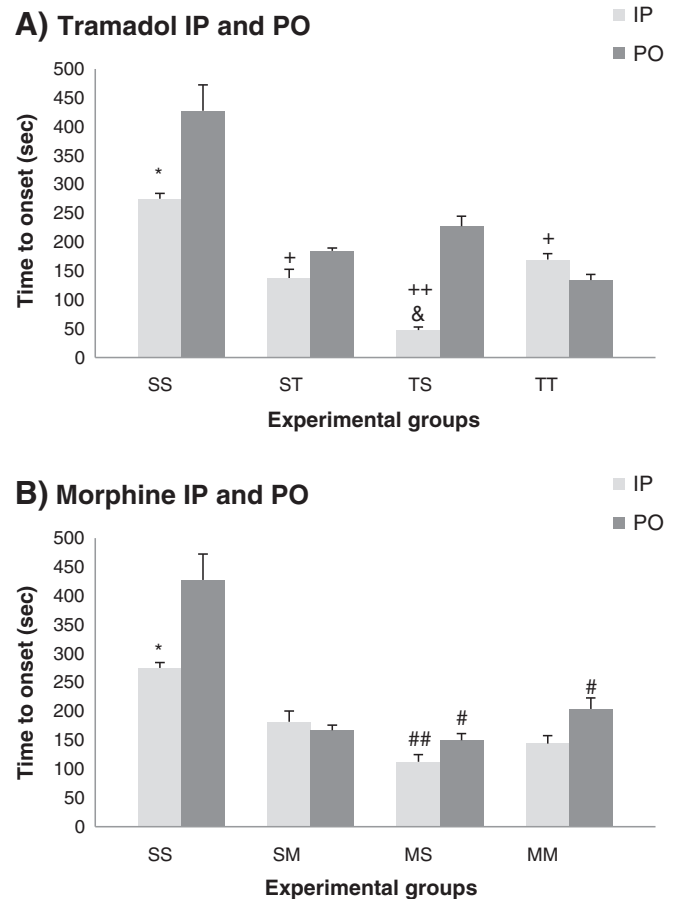
There were significant differences with respect to "time to onset of PTZ-induced seizures" between different tramadol IP (SS, ST, TS, and TT IP) groups [ $F(3, 22) = 98.3, p < 0.001$ ] and different tramadol PO (SS, ST, TS, and TT PO) groups [ $F(3, 20) = 26.8, p < 0.001$ ]. There was also a significant difference between different morphine IP (SS, SM, MS, and MM IP) groups [ $F(3, 20) = 26, p < 0.001$ ] and different morphine PO (SS, SM, MS, and MM PO) groups [ $F(3, 20) = 25.1, p < 0.001$ ]. Time to onset decreased in all morphine- and tramadol-treated rats compared with SS groups ( $p \leq 0.001$ ); however, TS and MS groups had the greatest effect, showing the shortest time to onset of PTZ-induced seizures.

Comparison of similar PO and IP groups showed significant differences between SS, TS, ST, TT, and MM PO with their similar IP groups (*t*-test,  $p < 0.05$ ) and between similar morphine and tramadol groups (TS IP, TS PO, and TT PO and their similar morphine groups;  $p < 0.05$ ). In summary, in most experimental groups, tramadol and morphine groups and also IP groups had shorter onset time compared with saline and PO groups, respectively. The related data are shown in Fig. 1.

### 3.2. Effects of morphine and tramadol on latency for myoclonic jerks

There were significant differences in terms of latency for myoclonic jerks between different tramadol IP (SS, ST, TS, and TT IP) groups [ $F(3, 22) = 20.6, p < 0.001$ ] and different tramadol PO (SS, ST, TS, and TT PO) groups [ $F(3, 20) = 141.4, p < 0.001$ ]. There was also a significant difference between different morphine IP (SS, SM, MS, and MM IP) groups [ $F(3, 20) = 13.1, p < 0.005$ ] and different morphine PO (SS, SM, MS, and MM PO) groups [ $F(3, 20) = 105.5, p < 0.001$ ]. In summary, latency for myoclonic jerks decreased in all morphine- and tramadol-treated rats compared with SS groups ( $p < 0.05$ , except SS IP with MS and ST IP); however, TS IP and MS PO groups had the greatest effect, showing the shortest latency for myoclonic jerks.

Comparison of similar PO and IP groups indicated a significant difference between SS, TS, and MS PO and their similar IP groups (*t*-test,  $p \leq 0.001$ ). There were also significant differences between similar morphine and tramadol groups (TS IP, TS PO, and TT PO and also their similar morphine groups;  $p < 0.05$ ). The related data are shown in Fig. 2.



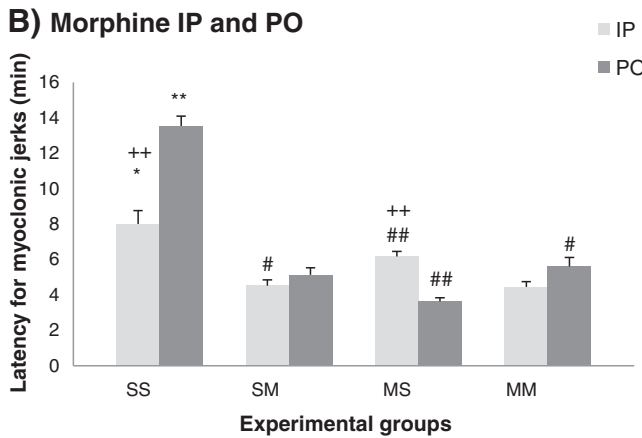
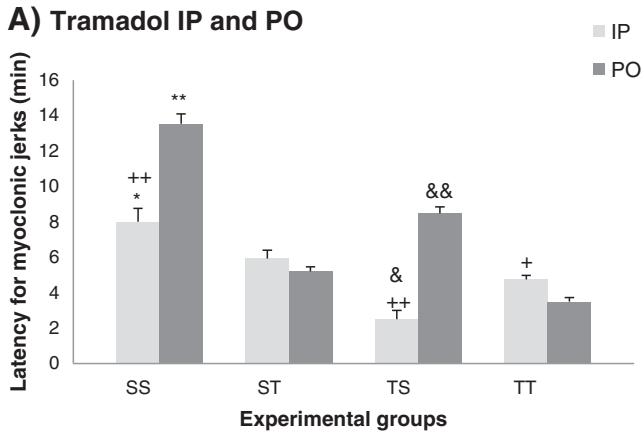
**Fig. 1.** Time to onset of PTZ-induced seizures; rats received morphine, tramadol, or saline on a daily basis for 15 consecutive days. For studying the long-term effect, on the 22nd day, rats of all groups were further divided into subgroups. Then, 30 min after the last drug administration, PTZ-induced seizures (80 mg/kg, IP) were assessed for 40 min. Panel A: There were significant differences between TT and ST IP with TS IP. & indicates  $p \leq 0.05$  [TS IP vs. ST IP and TT IP]. Panel B: # and ## indicate  $p \leq 0.05$  and  $p \leq 0.001$ , respectively, with their similar tramadol groups [MS IP vs. TS IP, MS PO vs. TS PO, and MM PO vs. TT PO]. Panels A and B: There were significant differences between SS IP and PO with other IP and PO groups in each panel. \* indicates  $p \leq 0.001$ ; + and ++ show  $p \leq 0.05$  and  $p \leq 0.001$ , respectively, with their similar PO group [SS IP vs. SS PO, ST IP vs. ST PO, TS IP vs. TS PO, and TT IP vs. TT PO].

### 3.3. Impact of morphine and tramadol on mortality rate

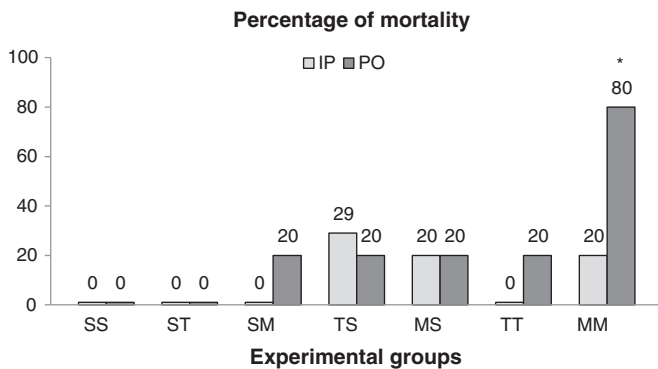
There was a significant difference in the percentage of mortality between MM PO and SS PO / and SS IP (Fisher's exact test,  $p < 0.05$ ). In the MM PO group, four (80%) out of five rats died because of PTZ-induced seizures, while no rats died in SS PO/ and SS IP groups. Therefore, the highest PTZ-induced mortality rate occurred in the MM PO group in comparison with other experimental groups (Fig. 3).

### 3.4. Effects of morphine and tramadol on percentage of level 4 behavior (turning over onto one side with tonic-clonic seizures)

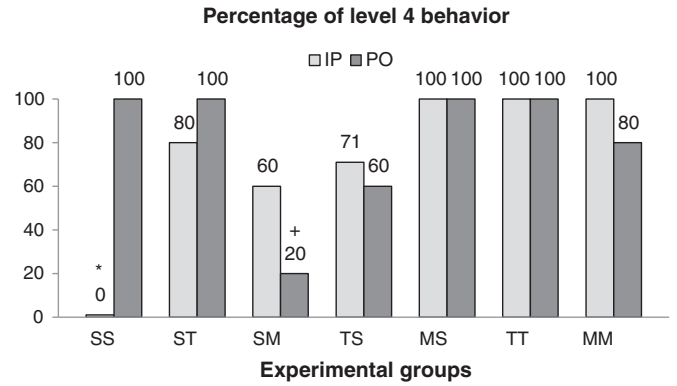
There were significant differences in the percentage of level 4 behavior between SS IP and other IP groups (except SM IP), between SS IP and SM PO groups with SS PO, and between SM PO and MS PO groups (Fisher's exact test,  $p < 0.05$ ). To sum up, tramadol and morphine groups had higher percentage of level 4 behavior compared with saline IP groups. There was not significant change between PO and IP administration routes in most of the experimental groups (Fig. 4).



**Fig. 2.** Latency for myoclonic jerks in PTZ-induced seizures; rats received morphine, tramadol, or saline on a daily basis for 15 consecutive days. For studying the long-term effect, on the 22nd day, rats of all groups were further divided into subgroups. Then, 30 min after the last drug administration, PTZ-induced seizures (80 mg/kg, IP) were assessed for 40 min. Panel A: There were significant differences between TS IP and PO with TT and ST IP and PO; & and && indicate  $p \leq 0.05$  and  $p \leq 0.001$ , respectively [TS IP vs. ST IP and TT IP; TS PO vs. ST PO and TT PO]. Panel B: # and ## show  $p \leq 0.05$  and  $p \leq 0.001$ , respectively, with their similar tramadol groups [SM IP vs. ST IP, MS IP vs. TS IP, MS PO vs. TS PO, and MM PO vs. TT PO]. Panels A and B: There were significant differences between SS IP and PO with other IP (except MS and ST IP) and PO groups in this panel. \* and \*\* indicate  $p < 0.05$  and  $p \leq 0.001$ , respectively. Also, + and ++ show  $p \leq 0.05$  and  $p \leq 0.001$ , respectively, with their similar PO group [SS IP vs. SS PO, TS IP vs. TS PO, TT IP vs. TT PO, and MS IP vs. MS PO].



**Fig. 3.** Percentage of mortality rate in PTZ-induced seizures; rats received morphine, tramadol, or saline on a daily basis for 15 consecutive days. For studying the long-term effect, on the 22nd day, rats of all groups were further divided into subgroups. Then, 30 min after the last drug administration, PTZ-induced seizures (80 mg/kg, IP) were assessed for 40 min. \* indicates  $p < 0.05$  with SS, ST (both IP and PO), and SM IP and TT IP.



**Fig. 4.** Percentage of level 4 behavior in PTZ-induced seizures; rats received morphine, tramadol, or saline on a daily basis for 15 consecutive days. For studying the long-term effect, on the 22nd day, rats of all groups were further divided into subgroups. Then, 30 min after the last drug administration, PTZ-induced seizures (80 mg/kg, IP) were assessed for 40 min. \* indicates  $p < 0.05$  with other IP groups (except SM); + indicates  $p < 0.05$  with SS PO and MS PO.

3.5. Effects of morphine and tramadol on duration of level 4 behavior

There were significant differences between different tramadol IP (SS, ST, TS, and TT IP) groups [ $F(3, 22) = 4.2, p < 0.05$ ] and different tramadol PO (SS, ST, TS, and TT PO) groups [ $F(3, 20) = 190, p < 0.001$ ]. There was also a significant difference between different morphine IP (SS, SM, MS, and MM IP) groups [ $F(3, 20) = 5.5, p < 0.01$ ] and different morphine PO (SS, SM, MS, and MM PO) groups [ $F(3, 20) = 6.9, p < 0.005$ ]. Data analysis also showed a significant difference between SS and ST IP and their similar PO groups ( $t$ -test,  $p \leq 0.001$ ). There were significant differences between similar morphine and tramadol groups (ST PO, TS PO, and TT IP with their similar morphine groups at  $p \leq 0.001, p \leq 0.001, \text{ and } p < 0.05$ , respectively). In sum, irrespective of the route of administration, tramadol groups had longer duration of level 4 behavior compared with morphine groups (Fig. 5).

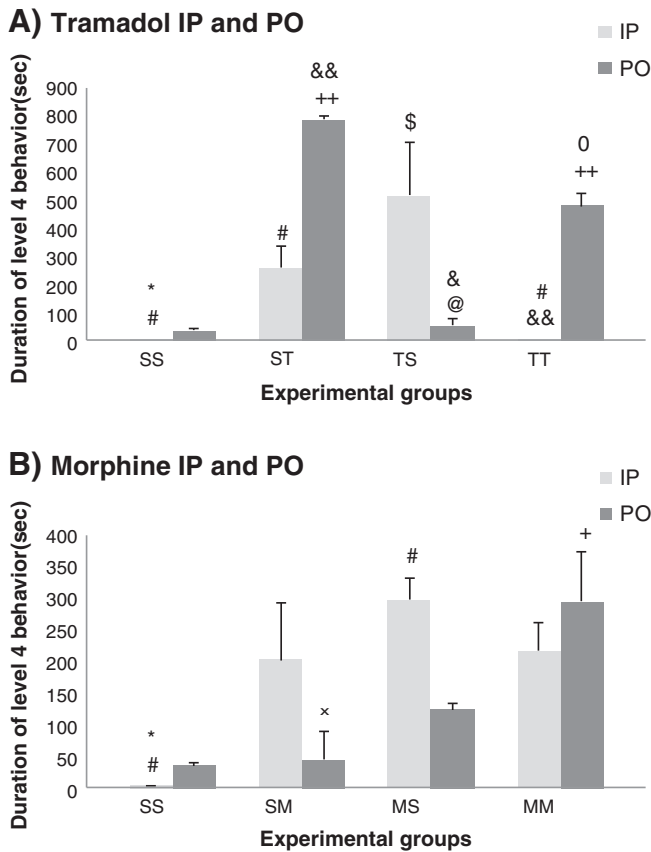
3.6. Effects of morphine and tramadol on duration of myoclonic jerks and tonic-clonic seizures

There were significant differences between different tramadol IP (SS, ST, TS, and TT IP) groups [ $F(3, 22) = 24.9, p < 0.001$ ] and different tramadol PO (SS, ST, TS, and TT PO) groups [ $F(3, 20) = 8.3, p = 0.001$ ]. Moreover, a significant difference was found between different morphine IP (SS, SM, MS, and MM IP) groups [ $F(3, 20) = 31.7, p < 0.001$ ] and different morphine PO (SS, SM, MS, and MM PO) groups [ $F(3, 20) = 37.9, p < 0.001$ ]. Data analysis of similar PO and IP groups demonstrated a significant difference between SS, MM, and TS PO and their similar IP groups ( $t$ -test,  $p < 0.005, p < 0.005, \text{ and } p \leq 0.001$ , respectively). Although there was a significant difference between some of tramadol and morphine groups (for example, TS IP and MS IP and TT PO and MM PO at  $p < 0.05 \text{ and } p < 0.005$ , respectively), other experimental groups did not reveal any significant differences in terms of this behavior (Fig. 6).

3.7. Effects of morphine and tramadol on severity of seizures

Severity of seizures was ranked based on the scale of 5 seizure behaviors in PTZ-treated animals plus death that was used by Saboory et al. ([69]). Concerning various seizure parameters, there were significant differences between different tramadol IP groups compared with SS IP group [ $F(3, 22) = 11.7, p < 0.001$ ] and different morphine IP groups compared with SS IP groups [ $F(3, 20) = 9.5, p < 0.001$ ]. There was no significant difference between morphine and tramadol and between different PO groups. The tramadol/saline group had the most





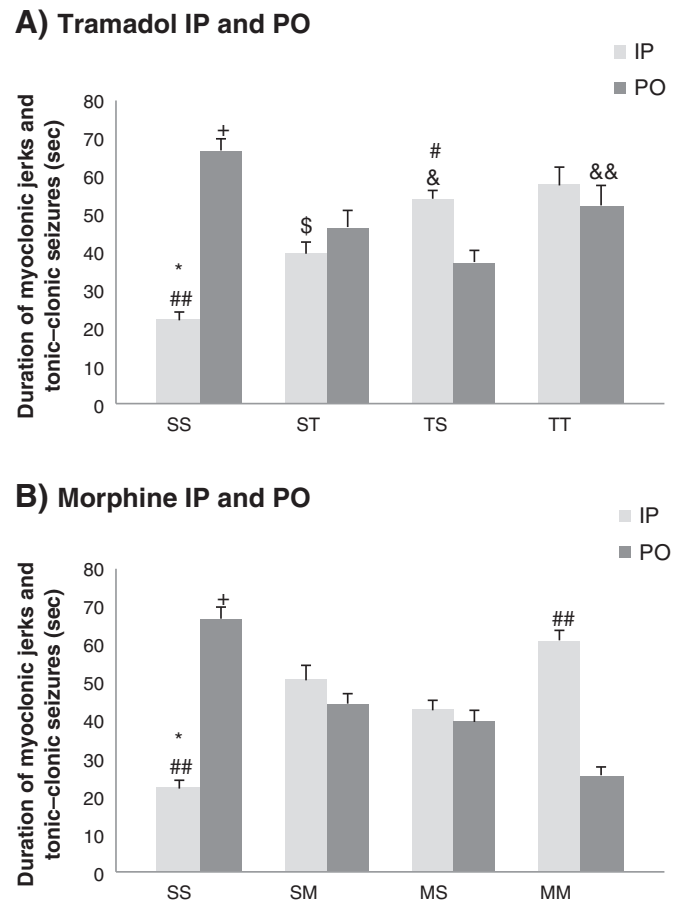
**Fig. 5.** Duration of level 4 behavior in PTZ-induced seizures; rats received morphine, tramadol, or saline on a daily basis for 15 consecutive days. For studying the long-term effect, on the 22nd day, rats of all groups were further divided into subgroups. Then, 30 min after the last drug administration, PTZ-induced seizures (80 mg/kg, IP) were assessed for 40 min. # indicates  $p \leq 0.001$  with similar PO; \* indicates  $p < 0.05$  with other IP groups (except TT and ST); & and && indicate  $p < 0.05$  and  $p \leq 0.001$ , respectively, with similar morphine group; × indicates  $p < 0.05$  with MM PO; + and ++ indicate  $p < 0.05$  and  $p \leq 0.001$ , respectively, with SS PO; @ indicates  $p \leq 0.001$  with ST PO; \$ indicates  $p < 0.05$  with TT IP; 0 indicates  $p \leq 0.001$  with TS and ST PO.

severe seizures in comparison with other groups. The related data are presented in Table 1.

#### 4. Discussion

In the present study, the facilitatory effects of morphine and tramadol on PTZ-induced epileptic behaviors and mortality rate were investigated using different methods of administration in adult rats. According to the findings, these drugs decreased time to onset and latency of myoclonic jerks. The number of myoclonic jerks and percentage of level 4 behavior were increased in all morphine and tramadol IP groups. Duration of myoclonic jerks and tonic-clonic seizures was also increased in acute and chronic morphine (IP or PO) and tramadol (IP) groups. Duration of level 4 behavior was increased in tramadol and morphine PO groups. Finally, seizure severity was significantly increased in all acute and chronic morphine and tramadol IP groups. Altogether, according to the abovementioned results, although morphine and tramadol affected some seizure behaviors in different ways, both acute and chronic administrations as well as their withdrawal and relapse potentiated the PTZ-induced seizures.

Design of this study was a particular schedule of drug administration, withdrawal, and then readministration, all of which led to the facilitation of seizures. Saline treatment followed by a single dose of drug (saline-withdrawal-drug) indicated the proconvulsant effect of the



**Fig. 6.** Duration of myoclonic jerks and tonic-clonic seizures in PTZ-induced seizures; rats received morphine, tramadol, or saline on a daily basis for 15 consecutive days. For studying the long-term effect, on the 22nd day, rats of all groups were further divided into subgroups. Then, 30 min after the last drug administration, PTZ-induced seizures (80 mg/kg, IP) were assessed for 40 min. # and ## indicate  $p < 0.005$  and  $p \leq 0.001$ , respectively, with similar PO group; \* indicates  $p < 0.05$  with ST IP and  $p \leq 0.001$  with other IP groups; & and && indicate  $p < 0.05$  and  $p < 0.005$  with similar morphine group, respectively; + indicates  $p < 0.05$  with other PO groups (except TT PO); \$ indicates  $p < 0.05$  with TT IP.

acute administration of drug. In a similar manner, the continuous drug treatment followed by a withdrawal period and then a single dose of saline (drug-withdrawal-saline) or drug (drug-withdrawal-drug) reconfirmed stable and long-term proconvulsant effects of chronic administration and withdrawal of drug, respectively. This finding was in agreement with that of the previous work on neonatal and prepubertal

**Table 1**

Comparison of the seizure severity after administration of 80-mg/kg PTZ in the rats that were pretreated with saline, morphine, or tramadol either IP or PO.

Groups	IP groups	PO groups
Saline/saline	1.30 ± 0.14	1.82 ± 0.14
Saline/tramadol	2.31 ± 0.22*,#	2.72 ± 0.15
Saline/morphine	2.32 ± 0.15*	2.40 ± 0.34
Tramadol/saline	3.18 ± 0.30**	2.16 ± 0.42
Morphine/saline	2.64 ± 0.22**	2.45 ± 0.28
Tramadol/tramadol	2.56 ± 0.13**	2.86 ± 0.31
Morphine/morphine	2.91 ± 0.35**	2.86 ± 0.32

Values represent the mean ± SEM in rats that received morphine, tramadol or saline daily for 15 consecutive days.

\* Indicates  $p < 0.005$  with saline/saline IP.

\*\* Indicates  $p \leq 0.001$  with saline/saline IP.

# Indicates  $p < 0.01$  with tramadol/saline IP.

rats, indicating that the chronic administration of morphine and tramadol had a long-term effect and led to increased severity of PTZ-induced seizures [45]. Continued activation of pharmacological opioid receptors has been proven to precipitate seizures via GABA inhibitory pathways [56,57]. Observations of the present work were also in line with those of the previous findings that indicated that morphine and tramadol could modulate seizure susceptibility [21,40,41,58,59] and show proconvulsant effects in higher doses [24,39,60]. Proconvulsant effects of  $\mu$ -opioid system activation have been reported in various seizure models induced by PTZ [24,61,62], bicuculline [63,64], NMDA [65], and pilocarpine [66], suggesting a more general modulatory influence on other seizure models. Since  $\mu$ -opioid system activation produces a similar phenotype of modulation in each seizure model [60], it is possible for opioids to target a common cellular mechanism that is prevalent in each model and encodes facilitatory effects in these models.

According to the present data, no significant differences in severity of seizures and most of other seizure parameters were observed between morphine and tramadol IP and PO administration routes. Route of administration of a given drug can have a significant influence upon the whole body and brain distribution. The highest levels of opioid receptor peptides in the brain have been reported 120 min after IP administration [67]. By contrast, brain levels of opioid peptides following PO administration have reached the peak 240 min after the administration [67]. According to the current data and irrespective of some differences in seizure parameters and severity between IP and PO administration routes in experimental groups, no significant changes were observed. In the schedule of chronic drug administration and withdrawal, there was enough time for morphine and tramadol brain distribution following both IP and PO administrations. Route of administration did not, therefore, impact differently on seizure behaviors. In the schedule of acute drug administration, a seizure was induced 30 min following drug administration and was monitored for 40 min. Therefore, it seems that, during this period, drugs might not reach the peak at the highest level in the brain following IP and PO administrations and could not be appropriately compared. A time point study will be useful for addressing this issue.

In this study, there was no significant difference in terms of seizure severity between morphine and tramadol. It has been previously demonstrated that morphine's antinociceptive effect is several times more powerful than that of tramadol. However, it seems that the anticonvulsant mechanism of action of these drugs (at least for PTZ-induced seizures) is compatible and might be different from their antinociception mechanisms. Tramadol has a dual mechanism of action through both  $\mu$ -opioid receptor-dependent and independent systems [39]. It has been reported that the tramadol-induced proconvulsant effect is through opioid-dependent gamma-aminobutyric acid inhibitory pathway [38]. Also, it has been expressed, pretreatment with gabapentin, a GABA-releasing agent, and administration of naloxone, a nonselective opioid receptor, markedly reduces tramadol-induced potentiation of PTZ seizures [68]. Overactivation of opioid receptors has been shown to precipitate seizure activity in various laboratory animals [60], which involves multiple opiate receptor-mediated mechanisms [22]. It appears that GABAergic systems may be of particular significance for elucidating varied effects of opioids on seizure susceptibility [23,56,57].

In conclusion, this study indicated that both morphine and tramadol had a proconvulsant effect on PTZ-induced seizures, and there was no significant difference between them in terms of seizure severity; this point might depend on their similar effects on GABAergic pathways. Also, the impact of morphine and tramadol on PTZ-induced seizures was stable after one-week withdrawal of these drugs, and there was no difference in terms of seizure severity between routes of administration. Since morphine and tramadol produce a similar phenotype of modulation in PTZ seizure model, these opiates may target a common cellular mechanism. While it has been hypothesized to be opioid-dependent inhibition of GABAergic pathways, further effort is required to establish the precise nature of their interaction.

## Ethical approval

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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## Conflict of interest statement

The authors have no conflicts of interest to declare regarding the study described in this article and the preparation of the article.

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