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Tramadol Effect on Morphine Dependency and Analgesia in Mice

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Abstract: This study was performed to investigate the effect of tramadol on morphine dependency and analgesia. Mice were divided into 5 groups, (1) Morphine-dependent, (2) Tramadol-dependent, (3) Morphine-dependent accompanied by saline, (4) Morphine-dependent accompanied by tramadol (50 mg kg^{-1}) and (5) Tramadol 30 min pretreatment of naloxone in the last day in morphine-dependent mice. Hot-plate, formalin and writhing tests were applied to investigate antinociceptive effect of tramadol in different doses (12.5, 25, 50 and 100 mg kg^{-1}). Latency time for jumping in group 4 (11.64±1.44 min) was less than group 1 (19.62±2.28 min) ($p<0.05$). The dose of 50 and 100 mg kg^{-1} of tramadol induced more tolerance in mice in hot-plate test. The most of this effect is for tramadol 100 mg kg^{-1} 30 min after beginning the test to be controlled ($p<0.05$). In formalin test tramadol 50 mg kg^{-1} in both acute (7.17±2.66 min) and chronic (19.5±9.22 min) phases showed the most effectiveness. In writhing test the most effective dose was 50 mg kg^{-1} of tramadol as well. It seems tramadol can increase the depth of morphine dependence in mice. Also, tramadol antinociceptive effect in high doses can appear the comparative effect with morphine in hot-plate, formalin and writhing analgesic models.

Key words: Tramadol, morphine, naloxone, dependence, jumping, hot-plate, formalin, Writhing test

INTRODUCTION

Tramadol is a centrally acting synthetic analgesic with both opioid and non-opioid properties (Lee *et al*., 1993; Raffa *et al*., 1992). It stimulates neuronal serotonin release and inhibits the presynaptic re-uptake of both noradrenaline (norepinephrine) and serotonin (Collart *et al*., 1993; Poulsen *et al*., 1996). Despite its long-term use, the understanding and prediction of the time course of its pharmacological effects are still hampered by the presence of active metabolites and the coexistence of opioid and nonopioid mechanisms. The affinity of tramadol for μ-opioid receptor is weak, approximately 10-fold less than that of codeine and 6000- fold less than that of morphine. Therefore, μ-opioid receptor activation appears to be only one component of the mechanism of action of Tramadol (Gillen *et al*., 2000; Minami *et al*., 2007). A further mode of tramadol action has been identified as the inhibition of the reuptake of monoamines, such as norepinephrine and serotonin, release from nerve endings. This inhibitory effect may also contribute to the analgesic effect of tramadol by inhibiting pain transmission in the central nervous system (Raffa *et al*., 1992; Reimann and Hennies, 1994). Although μ-opioid receptors and monoamine transporters are thought to be the sites of tramadol activity, additional sites probably exist, based on additional clinical and analgesic effects of tramadol. In animal models, tramadol also has an anti-inflammatory effect which is independent of prostaglandin inhibition (Buccellati *et al*., 2000). In such subjects, tramadol has little or no analgesic effect (Poulsen *et al*., 1996). Further biotransformation results in inactive metabolites which are excreted by the kidneys. A comparison of receptor site affinities and mono-amine re-uptake inhibition illustrates the unique combination of properties which underlie the action of tramadol, it is necessary to invoke synergism to explain its analgesic effect (Minami *et al*., 2007). Tramadol causes much less constipation and respiratory depression than equi-analgesic doses of morphine (Wilder-Smith and Bettiga, 1997). It has no effect on pressures in the biliary and pancreatic ducts (Wilder-Smith *et al*., 1999). By injection, tramadol is 1/10 as potent as morphine. By mouth, because of much better bio-availability, it is 1/5 as potent; it can be regarded as double strength codeine (Grond *et al*., 1995; Lehmann, 1994). The clinical use of morphine for long periods of time is limited by its propensity to cause tolerance and physical dependence after repeated administration. To overcome tolerance to the analgesic effects of morphine, higher doses are necessary for adequate pain relief but are often accompanied by undesirable physical dependence and

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side effects such as constipation, nausea and respiratory depression (Pasternak, 2001). The potential ability of tramadol to induce dependence has been preclinically evaluated in different animal species (Epstein *et al*., 2006; Matthiesen *et al*., 1998). Although, its dependence liability is also considerably less (Preston *et al*., 1991) and it is not a chronic disease (Radbruch *et al*., 1996). Tramadol in chronic use can induce some degree of drug dependence in animals (Miranda and Pinardi, 1998; Preston *et al*., 1991; Vickers *et al*., 1992). Although animal experiments did not reveal withdrawal reactions after chronic tramadol administration in rats (Miranda and Pinardi, 1998) and only mild to intermediate symptoms in monkeys (Epstein *et al*., 2006). Tolerance was not induced in arthritic rats, in contrast to nalbuphine, buprenorphine and morphine (Kayser *et al*., 1991). No cross tolerance between morphine and tramadol was observed in these animals and naloxone only partially reverses the analgesic effect of tramadol (Epstein *et al*., 2006). On the basis of this effect, its potency of dependence is less than morphine and it is possible that tramadol can decrease the intensity of the effect of morphine dependence. It can probably exert an effect on morphine dependence like methadone. It is supposed that tramadol can interfere in the morphine-dependence profile. This effect may help to withdraw morphine dependent subject when the depth of dependency is high. Also, we supposed that the dependence of the tramadol treatment might be lighter than morphine. On the basis of this hypothesis, we studied possibly interaction of tramadol in morphine dependent mice using jumping induced by naloxone in dependent animals. So, we wanted to survey possibly methadone like effect of tramadol if any and to compare the antinociceptive effect of tramadol with morphine in hot-plate, formalin and writhing analgesic models. This research was designed to study the effect of tramadol on morphine dependent mice and its effect on analgesia by three different evaluation methods.

MATERIALS AND METHODS

Animals: Male Albino mice, weighing 20-25 g, were used. Animals were housed at 22±2°C, on a 12 h light/dark cycle, with food and water *ad libitum*. Each experimental group consisted of minimum 12 animals. For evaluating the physical dependency, the mice were divided into five treatment groups [group 1: Morphine (alone) (n = 12), group 2: Morphine + Saline (n = 12), group 3: Morphine + Tramadol 30 min before Naloxone (n = 12)] for a period of 4 days, group 4: Morphine + Tramadol (n = 24), group 5: Tramadol (alone) (n = 12). Different doses of tramadol (12.5, 25, 50 and 100 mg kg^{-1}, i.p.) were given to test its antinociceptive effect in hot-plate, formalin and writhing tests. For evaluating the antinociception effect, the experimental group consisted of minimum 6 animals. All experiments were done under supervision of University ethical committee based on Helsinki declare in animal studies. The protocol was approved by the university ethical committee.

Drugs: Tramadol hydrochloride sulphate (WIEB pharm Co., Germany), naloxone hydrochloride and morphine (Tolid Daru, Tehran, Iran) were purchased. Before administration, all drugs were dissolved in 0.9% saline.

Tests: In this study two major types of evaluations include physical dependency and antinociception evaluating tests were used. For physical dependency jumping test and for antinociception effects of tramadol hot-plate, formalin and writhing test were applied.

Physical dependence evaluation

Jumping test: To test for physical dependence, morphine or tramadol-dependent mice were given naloxone (1 mg kg^{-1} s.c.) and immediately placed on a circular platform approximately 1.5 ft in height for no more than 30 min (Takemori and Sprague, 1978). The latency time for jumping, number of jumps and the amount of defecation and urination (DU) during that period in each group were evaluated. A trained observer who was blinded to experimental treatments reviewed the data.

Antinociceptive evaluation

Hot-plate test: The hot-plate test was assessed on groups of 6 to 12 mice. The temperature of a metal surface was maintained at 55±0.2°C. Latency to a discomfort reaction (licking paws or jumping) was determined before and after drug administration. The cut-off time was 40 sec. The latency was recorded before and 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 min following intraperitoneal administration of the drug. The prolongation of the latency times compared with the values of the control was used for statistical comparison. Control mice were given normal saline (10 mL kg^{-1}, i.p.) as reference drug (Rojas-Corrales *et al*., 2003).

Formalin test: In this study, formalin test is used previously published by Hunskaar *et al*. (1985) with slight modifications (Takeshita and Yamaguchi, 1995). Each mouse was placed in an observation chamber 5 min before the injection of diluted formalin to allow acclimation to the new environment. Ten milliliter of 1% formaldehyde in saline were administered into the left hind paw with a micro syringe. Each animal was then returned to the

observation chamber and nociceptive response was recorded for a period of 45 min. The summation of time (sec) spent in licking and biting of the paw that received injections during each 5 min block was measured as an indicator of the pain response. The duration of responses in the first 5 min and that from 15 to 45 min represents first and second phases, respectively. This test was performed in a temperature- and humidity-controlled (22±1°C, 55±5%) room.

Writhing test: Groups of 6 mice were used for controls and test subjects. Thirty min after the administration of the extract, the mice were given an intraperitoneal injection of 0.7% v/v acetic acid solution (volume of injection 0.1 mL/10 g). The mice were placed individually into glass beakers and five min were allowed to elapse. The number of writhes produced in these animals was counted for 30 min. For scoring purposes, a writhe is indicated by stretching of the abdomen with simultaneous stretching of at least one hind limb. Control mice were given normal saline (10 mL kg^{-1}, i.p.), as reference drugs. Naloxone (2 mg kg^{-1}, s.c.) was administered 15 min prior to the extracts or morphine injections (Rojas-Corrales *et al*., 2003).

Drugs administration: For physical dependency evaluation, 5 groups of mice were used. In all of them except group 2 were dependent to morphine (Marshall and Grahame-Smith, 1994), in which the mice were received subcutaneous (SC) injection of morphine three times daily (9 am, 13 pm and 17 pm) during 3 consecutive days with cumulative doses (50, 50 and 75 mg kg^{-1}). In group 4 mice were received tramadol (50 mg kg^{-1}, IP) 30 min before all morphine injections. A similar schedule was applied for group 3 with saline (10 mL kg^{-1}) (IP). The mice in group 1 were received morphine alone using Marshal Methods and the mice in group 5 were injected by single dose of tramadol (50 mg kg^{-1}) (IP) 30 min pretreatment of naloxone (1 mg kg^{-1}) (IP) in last day. Finally, group 2 mice were chronically injected by tramadol with Song and Takemori methods (Song and Takemori, 1992), in this procedure mice will be made chronically tolerant by IP injection of tramadol (100 mg kg^{-1}) (IP) three times daily (9 am, 13 pm and 17 pm) during 5 consecutive days. The physical dependence intensity was evaluated by measuring different signs (jumping latency period, number of jumps during 30 min, the weight of defecation/urination; DU) induced by naloxone (1 mg kg^{-1}) (IP) after the latest morphine dose in the final day of experiments. In hot-plate, formalin and writhing tests mice were pretreated by different doses of tramadol (12.5, 25, 50 and 100 mg kg^{-1}, i.p.) or morphine (10 mg kg^{-1}) and the mice then were evaluated.

Data analysis: After gathering the data, a code sheet for each mouse in each group was prepared. All results were presented as mean±SEM and for analysis of data of the study groups; one-way ANOVA post-hoc Tukey test was used. The difference between data was considered statistically significant at $p<0.05$ levels.

RESULTS

Physical dependence evaluation: Overall, the latency period for jumping in group 4 (morphine and tramadol) is less than group 1 (morphine alone) [(mean±SEM: 11.64±1.44 min vs. 19.62±2.04 min), ($p<0.05$)] and also group 2 (tramadol alone) is less than group 1 (morphine alone) [(5±0.99 min vs. 19.62±2.04 min), ($p<0.05$)]. There was not significantly difference between average numbers of jumps between five groups ($p>0.05$). Among the five groups of mice, the difference of DU in group 5 (single dose tramadol 30 min pretreatment of naloxone) was statistical significant (0.59±0.08 g) ($p<0.05$) (Table 1).

Antinociception evaluating tests

Hot-plate: At the first step of hot-plate test the latency time in mice pretreated with morphine 10 mg kg^{-1} (29.3±0.44 sec) is more than tramadol 25 mg kg^{-1} (14.7±0.52 sec) ($p<0.05$) and tramadol 50 mg kg^{-1} (16.7±1.57) ($p<0.05$). Latency time after 30 min in mice pretreated with naloxone 5 mg kg^{-1} + tramadol 50 mg kg^{-1} (18.2±1.83 sec) is less than other groups such as tramadol 100 mg kg^{-1} (40 sec) ($p<0.05$), tramadol 50 mg kg^{-1} (40 sec) ($p<0.05$), morphine 10 mg kg^{-1} (36.9±0.97 sec) ($p<0.05$). After 70 min the latency time of the mice that pretreated with tramadol 100 mg kg^{-1} (36.7±1.02 sec) is more than tramadol 25 mg kg^{-1} (26.7±1.79 sec) ($p<0.05$) (Table 2).

Table 1: Mean of jumping latency time, jumping count and the difference of the weight of defecation/urination (±SEM) for each group during jumping test as an evaluation of morphine and tramadol dependency effects in jumping test

Groups	Group of study	Jumping latency time (min)	Jumping count	DU difference[1] (g)
1	Morphine (only)	19.62±2.28	15.66±7.46	0.20±0.04
2	Tramadol (only)	5.00±0.99*	2.41±2.06	0.24±0.02
3	Morphine+Saline	16.62±1.77	14.41±4.20	0.15±0.02
4	Morphine+Tramadol	11.64±1.44**	28.11±9.02	0.22±0.03
5	Morphine+Tramadol 30 min before naloxone	12.22±1.44	34.83±9.83	0.59±0.08***

Values are significantly different in mean±SEM, *Significantly difference between group 2 and group 1 ($p<0.05$), **Significantly difference between group 4 and group 1 ($p<0.05$), ***Significantly difference between group 5 and all the other groups ($p<0.05$), [1]The Difference of the weight of defecation/urination for each group during jumping test

Table 2: Mean latency time (sec) ±SEM for saline, morphine and different doses of tramadol induced antinociception in hot-plate test in mice. Tramadol (IP), Morphine (SC) and Naloxone (IP) were administered concurrently and antinociception were used at different times (min), cut-off time was set at 40 sec. In each case, n = 6 to 12 mice per dose

	Time (min)										
Groups	0'	10'	20'	30'	40'	50'	60'	70'	80'	90'	100'
Saline	9.7±3.9	9.2±3.9	8.8±3.9	9.5±3.9	8.3±3.9	9.5±3.9	9.2±3.9	9.7±3.9	9.1±3.9	9.3±3.9	9.5±3.9
Tramadol 25 mg kg^{-1}	14.7±1.4	20.7±3.9	16.7±1.4	24.0±4.4	20.0±1.9	11.3±1.6	20.7±1.4	26.7±4.8	20.0±5.7	16.7±5.0	28.0±6.3
Tramadol 50 mg kg^{-1}	16.7±4.2	34.0±3.6	37.3±2.2	40.0±0.0	28.7±2.4	32.0±3.9	27.3±3.7	33.3±3.6	34.7±3.7	33.3±3.1	32.7±3.5
Tramadol 100 mg kg^{-1}	17.3±1.4	40.0±0.0	40.0±0.0	40.0±0.0	36.7±2.7	40.0±0.0	36.7±2.7	36.7±2.7[3]	32.7±3.7	38.7±1.1	30.7±2.9
Morphine 10 mg kg^{-1}	29.3±1.2[1]	40.0±0.0	39.1±0.9	36.9±2.6	20.0±2.3	19.6±0.9	20.0±1.1	17.3±1.3	13.8±1.7	24.0±4.0	17.8±1.8
Naloxone and Tramadol*	23.1±2.2	31.6±4.0	24.0±5.9	18.2±4.9[2]	22.7±4.0	21.3±6.0	20.9±4.8	20.9±4.5	19.6±3.2	18.2±3.5	17.7±3.5
Tramadol and Naloxone**	22.5±2.1	40.0±0.0	37.1±1.9	26.4±3.6	22.1±4.3	19.6±4.9	15.8±4.5	19.9±4.3	27.4±4.1	28.4±4.0	26.9±4.5

*Naloxone (5 mg kg^{-1}, 15 min before tramadol) and Tramadol (50 mg kg^{-1}, 30 min before test), **Tramadol (50 mg kg^{-1}, 30 min before injection) and Naloxone (5 mg kg^{-1}, before test)[1]Significantly difference between morphine 10 mg kg^{-1} and (tramadol 25 mg kg^{-1} - tramadol 50 mg kg^{-1}) just only after cut off time ($p<0.05$), [2]Significantly difference after 30 min between naloxone 5 mg kg^{-1} + tramadol 50 mg kg^{-1} and (tramadol 100 mg kg^{-1} - tramadol 50 mg kg^{-1} - morphine 10 mg kg^{-1}) ($p<0.05$), [3]Significantly difference after 70 min between tramadol 100 mg kg^{-1} and tramadol 25 mg kg^{-1} ($p<0.05$)

Table 3: Mean of licking (±SEM) as an evaluation of morphine and tramadol antinociceptive effects in formalin test

	Groups					
Time (min)	Saline	Morphine 10 mg kg^{-1}	Tramadol 12.5 mg kg^{-1}	Tramadol 25 mg kg^{-1}	Tramadol 50 mg kg^{-1}	Tra 50 mg kg^{-1} and Nal 5 mg kg^{-1}*
0-5	100.3±25.27	0.0	112.4±22.45	18.0±9.24	0.4±0.36	7.2±2.66
15-40	472.0±39.80	0.5±0.34	553.2±137.04	203.8±42.78	27.4±17.66	19.5±9.22

*Tramadol 50 mg kg^{-1} (30 min before injection) and Naloxone 5 mg kg^{-1} (before injection)

Table 4: Mean of body stretching (±SEM) as an evaluation of morphine and tramadol antinociceptive effects in writhing test

	Groups					
Time (min)	Saline	Morphine 10 mg kg^{-1}	Tramadol 12.5 mg kg^{-1}	Tramadol 25 mg kg^{-1}	Tramadol 50 mg kg^{-1}	Tra 5 mg kg^{-1} and Nal 50 mg kg^{-1}*
Body stretching	53.9±11.51	0.66±0.4	24.5±11.88	17.0±5.2	1.33±0.61	6.5±5.55

*Naloxone 5 mg kg^{-1} (45 min before beginning of the test) and Tramadol 50 mg kg^{-1} (15 min after Naloxone injection)

Formalin test: Formalin test results indicate that the most number of liking in both 0-5 and 15-40 min is for the mice was pretreated by tramadol 12.5 mg kg^{-1} (112.4±22.47) and saline (100.25±24.93) that significantly significant with 25, 50 mg kg^{-1} and morphine 10 mg kg^{-1} ($p<0.05$) (Table 3).

Writhing test: The results of writhing test in different groups indicate that the most of body stretching due to acetic acid injection is beyond for saline group and it has statistically significant difference to the other group ($p<0.05$) (Table 4).

DISCUSSION

According to this study, tramadol reduced jumping latency period and increased the number of jumps induced by naloxone in morphine dependent mice. On the other hand, tramadol can increase the depth of morphine dependency in mice. It seems that, it may be due to the effect of tramadol on the opioid receptors (Raffa *et al*., 1992). However, the affinity of tramadol is approximately 10- fold less potent than codeine in inhibiting μ-opioid binding, 60- fold weaker than d-propoxyphen, 1000-fold weaker than methadone and 6000-fold weaker than morphine. In fact the affinity of tramadol for opioid receptors appears to be insufficient to account for its efficacy and potency (Hennies *et al*., 1988; Raffa *et al*., 1992). Although, its affinity for μ_1 receptor is less than morphine (Miranda and Pinardi, 1998), but the mass effect of tramadol doses, can induce a competitive interaction with the morphine molecules for affect on its specific receptor (Raffa *et al*., 1992). This may be due to why the tramadol can reduce the jumping latency period by naloxone and increase the number of jumps. Thus, affinity alone is not sufficient to account for analgesic action of tramadol. The main metabolite of tramadol, o-desmethyl tramadol, binds with about 300- fold higher affinity than the parent compound, but this is still much weaker than the affinity of morphine (Frink *et al*., 1996; Hennies *et al*.,

1988). The increase in subjective and objective pain thresholds induced by tramadol contrast with those of other opioids in that they are only partially blocked by opioid antagonist naloxone (Gillen *et al*., 2000). It seems that this displacement effect of tramadol indirectly can increase the naloxone effect.

There has been some controversy regarding the dependence-liability of tramadol. The results of these studies have been mixed. Miranda and Pinardi administered tramadol (39.1 or 100 mg kg^{-1}; s.c.) three times daily for 5 days to mice and then tested them for tolerance in an experimental pain model (the acetic acid writhing test) and for physical dependence by injection with naloxone (1 mg kg^{-1}; i.p.) (Miranda and Pinardi, 1998). There was no evidence of tolerance to the antinociceptive response to the ED_{50} dose (7.82 mg kg^{-1}) of tramadol and there were few or no signs of withdrawal after administration of naloxone at either dose of tramadol. In contrast, a control group that received an identical regimen of morphine (1.05 or 100 mg kg^{-1}) injections showed significant tolerance to the morphine ED50 dose (0.21 mg kg^{-1}) and showed opiate-withdrawal signs on administration of naloxone. Almost no cross-tolerance was demonstrated: the antinociceptive response to tramadol was unchanged in the morphine-treated group and there was only a trend for decreased response to morphine in the tramadol-treated group. Thus, tramadol produced neither tolerance nor physical dependence in mice. Similarly, Murano evaluated tolerance and physical dependence in rats treated with up to 160 mg/kg/day in four divided s.c. injections. Tolerance to tramadol's antinociceptive effects was observed, but there was no evidence of physical dependence as indicated by weight loss following abrupt discontinuation of tramadol administration or following administration of levallorphan (Murano *et al*., 1978).

Some studies has tried to promote it as an opioid with little risk of dependence, claiming little evidence of such dependence in their clinical trials (Rossi, 2004). They offered the theory that, since the μ1 receptor metabolite is the principal agonist at μ-opioid receptors, the delayed agonist activity reduces the dependence-liability (Rossi, 2004). O-desmethyl tramadol (one of the main metabolites of tramadol) inhibits functions of μ1 receptors but has little effect on those of μ3 receptors (Nakamura *et al*., 2005). However, some evidence of physical dependence was detected in rats receiving tramadol orally (50 mg/ kg/day) and subjected to 24 h withdrawal with or without injection of naloxone (Nickel and Aledter, 1987). Similarly, there was some evidence of withdrawal in eight rhesus monkeys receiving tramadol four times a day (32–96 mg kg/day; s.c.) for 59 days: although few or no withdrawal signs were seen when naloxone (1.0 mg kg^{-1}; s.c.) was administered on four occasions during the administration period, withdrawal signs did emerge in the 5 days after tramadol was discontinued. These signs were graded as only mild (or, after the highest dose regimen, intermediate), not progressing to such severe signs as vomiting or diarrhea (Yanagita, 1978). In a concurrently run experiment, four rhesus monkeys self-administering tramadol for 4-6 weeks and administered naloxone (1.0 mg kg^{-1}; s.c.) at weeks 2 and 4 showed only mild-to-moderate withdrawal signs. The author concluded that the physical-dependence potential of tramadol is lower than that of pentazocine (Yanagita, 1978).

No antagonist activity has been demonstrated for tramadol in laboratory animals. Tramadol had only additive effects in an analgesic assay when combined with low doses of morphine and did not precipitate withdrawal jumping in morphine-dependent mice (Friderichs *et al*., 1978). Also, in animal models even high doses of naloxone (up to 8 mg kg^{-1} subcutaneously in mice) did not block tramadol-induced nociception completely (Collier *et al*., 1968) and in human volunteers only one third of tramadol-induced analgesia was inhibited by naloxone (Collart *et al*., 1993). This low range of affinity for opioid receptors appears as insufficient to explain its antinociceptive activity and it has been proposed that inhibition of neuronal uptake of noradrenaline and serotonin may contribute to the tramadol antinociceptive efficacy (Driessen and Reimann, 1992; Henies *et al*., 1982; Raffa *et al*., 1992). In summary, results of animal studies suggest that tramadol is an atypical opioid analgesic. It has some abuse potential, but, based on the self-administration studies in monkeys, less than that of prototypic opioids such as morphine. The evidence for physical-dependence capacity is mixed; withdrawal was not detected in mice, withdrawal was not detected consistently in rats and only mild-to-moderate withdrawal was detected in rhesus monkeys.

In present study, single dose of tramadol 30 min pretreatment of naloxone in withdrawal test in last day could increase the weight of defecation/urination (DU) in morphine dependent mice that induced by naloxone ($p<0.05$). This finding is consistent with Pandita *et al*. (2003) study which have shown that tramadol can increase the amount of mice micturition (Pandita *et al*., 2003). This effect may be due to the displacement effect of tramadol on opioid receptors and inhibits reuptake of 5-HT and noradrenaline. Opioid receptor-mediated inhibition of micturition can be caused by stimulation of μ and δ-opioid receptors (Dray and Metsch, 1984; Hisamitsu and de Groat, 1984; Kontani and Kawabata, 1988; Shimizu *et al*., 2000). Thus, administration of opioid

receptor active drugs systemically (Kontani and Kawabata, 1988; Sillen and Rubenson, 1986), intrathecally (Dray and Metsch, 1984; Durant and Yaksh, 1988; Hisamitsu and de Groat, 1984; Igawa *et al*., 1993) and intracerebroventricularly (Dray and Nunan, 1987; Hisamitsu and de Groat, 1984; Kontani and Kawabata, 1988; Sillen and Rubenson, 1986) inhibits micturition. The main site for inhibitory effects via opioid receptor stimulation is likely to be within the central nervous system (Dray and Metsch, 1984b). Pretreatment with naloxone abolished the effects of tramadol on micturition volume and attenuated the effects on micturition threshold pressure. This suggests that μ-opioid receptor activation plays a major role for tramadol causes an increase in both threshold pressure and bladder storage capacity, without impairing bladder emptying (Pandita *et al*., 2003). Furthermore, in vitro naloxone may facilitate electrically induced contractile activity of rat bladder strips (Berggren *et al*., 1991). Thus, it cannot be completely excluded that peripheral opioid receptor stimulation influences micturition.

The results in hot-plate test indicate that administration of high dose tramadol can induce similar antinociception effect in first 30 min like morphine and at least antinocicepton effect acquired after low dose administration of tramadol. These findings confirm dose related antinociception effect of tramadol (Raffa *et al*., 1992). After 60 min tramadol analgesia in varying doses is approximately permanent but morphine induce analgesia is decreased. However the affinity of tramadol is less than morphine in inhibiting μ opioid receptor (Hennies *et al*., 1988) but it is may be due to tramadol effects in presynaptic re-uptake inhibition of both norepinephrine and serotonin (Collart *et al*., 1993; Poulsen *et al*., 1996).

The results in formalin test indicate that both morphine and tramadol in high dose can induce similar antinociceptin effects and after a period of 15-40 min that inflammatory events causes pain in mice. It is interesting that high dose of tramadol like morphine can relief this pain in mice. This finding is consistent with Buccellati *et al*. (2000) study which has shown that tramadol has an anti-inflammatory activity that is not related to direct inhibitory action of prostaglandins. In writhing test results indicate tramadol comparable dose-related anti nociception effects with morphine too and it is consistent with pervious studies (Oliva *et al*., 2002).

This study finding shows that in all of the analgesic evaluation methods tramadol in high dose increases the antinocicepeton in mice and even it is comparable with morphine. The antinociceptive activity of tramadol in the mouse tail flick test is blocked by opioid antagonist (Friderichs *et al*., 1978) suggesting that tramadol-induced antinociception is mediated via opioid (naloxone-sensitive) receptors.

CONCLUSION

In conclusion, according to the results, if tramadol and morphine administrated together in a long-term period, the effect of morphine on its receptors will be handled and also in this group, withdrawal syndrome after naloxone administration will be appeared earlier than another group. On the other hand, pretreatment of tramadol in morphine dependent mice causes to increase morphine withdrawal syndrome intensity. It can be supposed that tramadol effect in reducing the increase in the depth of morphine dependency and high dose administration of tramadol can induce a comparable analgesia with morphine in mice.

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