Characterization of Tramadol Abuse at Different Dose Levels on Nociceptive Pain Thresholds and Immune Response

Abd El-Hamid Mohamed Elwy[1] and GhadaTabl[2]

[1]Forensic Medicine and Clinical Toxicology Department, Faculty of Medicine, Tanta University, Egypt.
[2]Zoology Department –Faculty of Science, Tanta University, Egypt.
ghada_tabl@yahoo.com

Abstract: Introduction: Pain management is a global challenge to clinicians. Pain relief has been put forward as an ethical obligation of clinicians and fundamental human rights. The World Health Organization has disseminated guidelines on pain management and advocated for the use of analgesics including opioid. Tissue injury or the presence of foreign materials initiates a series of pathophysiological events that may manifest as inflammatory pain.During inflammation response, several proinflammatory mediators are released, including IL-1, IL-6 and TNFα. These cytokines play major roles in initiation and amplification of inflammatory process. It is possible thatchoice of drug modulates beneficial immune response.It has been demonstrated that tramadol can contribute to beneficial effects. As, compounds known to block monoamine uptake, potentially have the antinociceptive effects of opioid including tramadol, the antinociception potency and profile of tramadol may derive from its combined opioid binding activity and inhibition of monoamine uptake. **Objective:** Carried out to evaluate the involvement of tramadol as effective analgesic on nociceptive thresholds at different dose levels (acute & chronic) and evaluate the potential immunological effects of this drug by determining IL-1, IL-6 and TNFα. **Methods:**152 male adult albino rats, scheduled for, 1- Evaluation of the antinociceptive effect of tramadol, by using two different stimulation tests(hot- plate&formalin tests) were applied to the groups subjected to the evaluation of antinociceptive activities of tramadol at different dose levels (acute & chronic).2-plantar incision, rats were divided into three subgroups (n=8 in each group). Rats in subgroup one, received anesthesia with no incision (sham control operation). Rats in subgroup two, plantar incision, without tramadol treatment. Rats in subgroup three received tramadol (36mg/kg) after plantar incision, as plantar incision induces heat hyperalgesia, heat hyperalgesia is assessed by measuring the heat threshold of rats to heat stimulation, by applying radiant heat source to the middle of the incision. The selected time point of 2 hours postoperatively was found to be adequate for the assessment of analgesic drug properties on incisional pain. **Results:** The latency period of rats subjected to hot-plate test was significantly decreasedwhereas, the number (no) of licking and biting of lesion paw edema in group subjected to formalin test, was significantly increased.The withdrawal latency period (W.L.P) of rat to heat stimulation,**(** which is applied to middle of the plantar incision using a focused radiant heat source**)** was significantly decreased, on the other hand the cytokines production were significantly elevated. These changes were reversed after tramadol treatment compared to control values. **Conclusion:** Considering, analgesic and immunosuppressive effect, tramadol treatment may be a drug of choice for treatment of acute and chronic pain particularly in patients with compromised immunity.

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1.Introduction

Pain is the oldest medical problem since the origin of humanity and remains a global public health issue. Pain relief has been put forward as an ethical obligation of clinicians and fundamental human rights. However, pain management is challenging, because the pathophysiology of pain is complex and, not completely understood (**Pergolizzi*et al.*,2012).** The world health organization **(WHO, 2000**; **2012)** has disseminated guidelines on pain management and advocated for the use of analgesics, including opioid. According **to Rafati(2006)** analgesics are among the most popular drugs abused.

Inflammation is a complex biological response of the body to cell damage and vascularized tissue **(Ferrero-Miliani*et al.*,2007; Hussein *et al.*,2012).** Once an inflammatory process begins, the mechanisms that perpetuate and amplify it are seemed to involve an ongoing imbalance between pro-and anti-inflammatory factors,central to this process are cytokines, particularly tumor necrosis factor (TNF-α) (**McColl, 2004).** During an inflammatory responses, several pro-inflammatory mediators are released, including interleukins, IL-1, IL-6, IL-12 and TNFα, as well as cylooxygenase-2 (Cox-2) **(Mueller *et al.*, 2010)** these cytokines play major roles in initiation and amplification of inflammatory process **(Colixto*et***

***al.,* 2004).** However, it can be pathogenic when it is produced excessively **(Xiong*et al.*,2000).** Acute inflammation is the body's primary response to injurious stimuli **(Gyurkovaska*et al.*,2011).**

Cytokines play a pivotal role in coordination of immune responses **(Gaspani*et al.*,2002; Niemand*et al.*,2003).** Surgical trauma and anesthesia are associated with a complex dysregulation of the immune system and activation of both proinflammatory and anti-inflammatory responses **(Salo, 1992).** Interleukins, IL-1, IL-6 and TNF-α have local and systemic effects that may limit injury and the spread of infection and provide a suitable environment for tissue healing and repair **(Sheeran and Hall, 1997).** It is possible that choice of drug modulates beneficial immune responses. It has been demonstrated that tramadol can contribute to beneficial effects on immune function. **(Sacerdote*et al.*,1997; 1999; 2000).**

Pain management is a global challenge to clinicians and, despite the plethora of evidence based guidelines; all analgesic options must be individually assessed and weighed for specific risks and benefits in a given patient.

Tramadol

Tramadol is a novel centrally, synthetic, analgesic with both opioid and non-opioid mechanisms responsible for its clinical effects. Structurally it is not an opiate, it exhibits some opioid characteristics. Like opioid it binds to mu (μ) opioid receptors, although very weakly **(Raffa*et al.*,1992; Raffa, 2001).** Clinically active tramadol is a racemic mixture of two enantiomers, (+) tramadol enantiomer and (–) tramadol enantiomer that have dual and complementary mechanisms of action. The (+) tramadol enantiomer is a selective agonist for the mu-opioid receptors, and inhibits serotonin reuptake, whereas the (–) tramadol enantiomer mainly inhibits norepinephrine re-uptake, i.e. tramadol has weak agnostic effect at mu-opioid receptors as well as inhibition of monoamines reuptake (serotonin and norepinephrine) **(Faron-Górecka*et al.*,2004**). These are believed to primarily contribute to its antinociceptive effects **(Hara *et al.*,2005).** According to **Berrocoso*et al.*(2007)** compounds known to block monoamine uptake, potentially have the antinociceptive effects for opioid including tramadol. Antinociceptive potency and profile of tramadol may derive from its combined opioid binding activity and inhibition of monoamine uptake **(Sacerdote*et al.*,1997**). Opioid analgesics are commonly used for the treatment of both acute and chronic pain. Some studies argued that they also cause suppression to immune system **(Clarck*etal.*,2007)** and **(Liu *et al.*,2008).** In addition, tramadol hydrochloride is one of the drugs that is used in treatment of opiate withdrawal **(Tamaskar*et al.*,2003)** and may be especially useful in outpatient detoxification **(Sobe*et al.*,2003).** Tramadol is rapidly and almost completely absorbed with a peak blood concentration, within 2 hours. (**Pothiawala, 2011).**

Tramadol has been established as adjunct to non-steroidal anti-inflammatory drugs in the treatment of moderate post-operative pain **(Le Rouxand Coetzee,2000).**

Objective of the current study: is to focus on the applicability and efficacy of tramadol as effective analgesic drug on nociceptive thresholds and on the potential immunological effects. Thus, we examined the nociceptive effect of tramadol, by using two different stimulation tests and evaluate its effect on proinflammatory responses by measuring cytokine levels; IL-1, IL-6 and TNF-α in rat model.

2.Material and Methods

Animals and laboratory

A total of **152** male adult albino rats with initial body weight ranging from 170-200 mg were used. They were obtained from Breeding Unit of the Egyptian Organization for Vaccine and Biological Preparations. All rats were fed *ad libitum* with standard diet and allowed free access of water and housed under standard laboratory conditions. Animal experimentations were carried out in an ethical manner following guide lines for scientific research. Rats were housed at a constant room temperature of 22°C with a 12-h. alternating light – dark cycle. After the surgical procedure, rats were housed and isolated in a large cage, the floor was covered with clean sawdust free from dirt as bedding material.The sawdust was changed twice a week to keep the animals dry throughout the period of the study and to minimize the possibility of painful mechanical stimulation. Using rats as animal models give us a good opportunity to know the real effect of the given drug.

Animals and experimental design

Rats were divided into 3 main groups

Group I: Control groups(n =56)have been always kept in parallel with experimental groups and subjected to simultaneous investigations.

Animals (controls) were divided into four subgroups.

- **Subgroup one** (n = 16): Controls for antinociception evaluations of tramadol acute dose group,8 rats for hot-plate test and8 rats for formalin test. This was carried out by injecting ratsintraperitoneal (i.p.) with normal saline 0.9% in a dose corresponding to tramadol treated groups.
- **Subgroup two** (n=16)Controls for antinociception evaluations of tramadol chronic dose group, Eight rats (8) for hot-plate test and eight rats (8) for formalin test.

- **Subgroup three** (n=16): controls for 96-h. withdrawal group (8 rats for hot-plate test and8 rats for formalin test).
- **Subgroup four** (n = 8): Control for plantar incisionand assessment of cytokines evaluation. Animals received a sham operation that consists of anesthesia 300mg/kg chloral hydrate (i.p.) and sterile preparation of the hind paw without incision. Animals were injected with normal saline 0.9% before sham operation. **(Brennan et al., 1996)**

Group II: Tramadol injected groups (n = 72): They were divided into three subdivided treated groups:

- **Subtreated group one**: Tramadol acute dose group (n = 16). Rats were injected (i.p.) with a single dose of 300 mg/kg b.w of tramadol (LD_{50}) according to **Matthiesen*et al.***(1998). They were tested for their nociceptive thresholds as evidenced by evaluating the paw withdrawal latency time (8 rats) (hote-plate test) and number of licking and biting of lesion paw edema in formalin test (8 rats).
- **Subtreated group two :**Tramadol chronic administration groups (n=48). To evaluate the repeated increasing cumulative doses effect on nociceptive response.. In these experiments,all rats were injected i.p. with a single daily therapeutic dose of 36 mg/kg tramadol for 7 consecutive days. On day 7, sixteen (16) rats were selected and tested for their responses to heat threshold;(8) rats for withdrawal latencies in hot-plate test and (8)rats for licking and biting of lesion paw edema in formalin test. The dose was increased to the double initial calculated therapeutic dose 72 mg/kg tramadol. The remaining rats were injected (i.p.) with a daily dose of 72 mg/kg tramadol for another 7 consecutive days. On day 14th, sixteen rats were selected and examined for their threshold responses hot-plate and formalin tests. The remaining sixteen rats were injected i.p. with 3 folds of the initial calculated therapeutic dose for another 7 consecutive days, then on day 21st, rats were tested for their nociception threshold. At the end of each experimental period (7, 14, 21 days), rats were tested for the latencies to a discomfort reaction; the prolongation of the latency periods were recorded in hot-plate tested group and nociceptive responses; licking & biting paw edema were estimated for a period of 30 min. during formalin test. The duration period was chosen according to period previously adopted by several investigates that ranged from single dose up to 21 days, **(Faron-Górecka*et al.*,2004; Filip*et al.*,2004; Atici*et al.*,, 2004 and Sepulveda *et al.*,2004)**.

Subtreated group III (n = 16). 96-hr. Withdrawal group.

In this experiment, rats (16) were injected i.p. with a single daily therapeutic dose of tramadol for 21 consecutive days. Rats were kept with no drug treatment over a withdrawal time of 96-h after the last given dose. Rats were tested for their nociceptive responses as previously described. **(Sepulveda *et al.*, 2004)**.

Group III: Plantar incision group and assessment of cytokines production (n=16): Eight rats for plantar incision group,andeight rats received tramadol after incision.

Drug under investigation; Tramadol hydrochloride ampoules, October pharma. Product S.A. Tramadol was used, because it is among the most commonly abused drug.

Dosage: All the tested doses, however, were mainly attributed to the recorded therapeutic dose of the drug. In the present experimental investigation, the adopted experimental dose level has been calculated as equivalent of human therapeutic dose according to **Paget and Barnes equation, (1964**)and **Samaka calculation, (2012)** the therapeutic dose for rat weighting 200 gm. was 7.2mg.

Methods and techniques

Experimental procedures: for nociceptive threshold's evaluation, includes two tests:

- **Hot-plate test**. The temperature of metal surface was maintained at 50°C. Latency to a discomfort reaction was determined before and after the drug administration. The cut- off time was 50 sec. According to **Tayebi*et al.*(2008)** with some modification.
- **Formalin test**. Formalin test is used previously by **Hunskaar*et al.*(1985)** with slight modification **(Takeshita and Yamaguchi, 1995).** Each rat was placed in an observed chamber 5 minutes before the injection of dilute 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 30 min according to **Tayebi*et al.*(2008)** with some modification.

Plantar incision procedure

According to **Brennan *et al.*(1996),** rats were anesthetized intraperitoneally (i.p.)with 300 mg/kg Choral hydrat, a 1 cm longitudinal incision was made through skin and fascia of the planter aspect of the left hind paw including the underlying muscles. The skin was sewed up with two mattress sutures and the wound was covered with iodine. Heat hyperalgesia is assessed by measuring withdrawal latency of rats to heat

stimulation which is applied to middle of incision using a focused radiant heat source **(Zahn and Brennan, 1999).** At the end of the previous experiments, rats were sacrificed quickly with the least disturbance by fast decapitation. Rats received anesthesia without incision was used as baseline. All blood samples were centrifuged at 1500 g (3000 rpm)for 10 min. and the separated sera was stored at-20 until use.

Measurement of IL-1, IL-6, and TNFα.

By enzyme-linked immunosorbent assay (ELISA). Serum IL-1, IL-6, and TNF-α levels were measured by using a rat specific ELISA kit. (IBL-International GmbH, Hamburg Germany) following the manufacturer's protocol.

Data analysis

Data were presented as mean ± SE. Data were analyzed using the computer program of SPSS. All statements of significance were based on probability of $P \leq 0.05$ was considered to be significant.

3.Results

The current study has been conducted to give aninformationabout the antinociceptive effects accompanying either acute or chronic administration of tramadol in a mammalian experimental model as well as, its immunosuppressiveeffects by measuring pro-inflammatory cytokines, IL-1, IL-6, and TNF-α. Rats repeatedly given tramadol for a long period of time at different dose levels, showed obvious gain in body weight. In addition, at the higher tested doses, some of the injected rats displayed noticeable behavioral neurotoxicological changes as excitation and disturbances of the locomotors changes as excitation and disturbances of the locomotors activity.

Tramadol antinociception evaluation tests

The effect of tramadol on the thermal hyperalgesia were assessed by measuring paw withdrawal latency (P.W.L.) in the rat model by:

a- Hot-plate-test (latency time).

b- Formalin test (number of licking and biting).

Effect of Acute dose of tramadol, 300mg/Kg (LD_{50}).

As illustrated in (Table 1 & Figure 1) a significant increase in the mean latency time (hot-plate group) $p \leq 0.05$, the mean values were 7.25 ± 1.04 and 37.0±1.85 for control and tramadol treated groups respectively**.**On the other hand, the number of licking and biting of lesion paw edema were significantly decreased 25.13±2.70 after tramadol treatment as compared to control value 79.50±13.33 $p \leq 0.05$.

Cumulative effect of increasing tramadol doses of either withdrawal latency time (hot plate- test) and no. of licking& biting (formalin- test)

As regards, the cumulative increasing tramadol doses 36, 75 and 150mg/kg b.w.(Table 2 &Figure 2). The data recorded on the 7th day of tramadol administration showed significant increase $p \leq 0.05$ in latency time, the mean values were (7.63±0.52) and (31.50±1.93) for control and tramadol treated groups respectively, followed by another significant increase on the day 14th $p \leq$ 0.05with mean (39.0±0.93**)** compared to the day 7th (31.50±1.93) and control value(7.63±0.52). On the day 21st, the data obtained showed insignificant change in the paw latency time compared to that of day 7th and day 14th of drug administration, but there was significant increase in the paw latency time when comparing the 21st group (150mg/kg) with that of control group.The mean values were (7.63±0.52 and 22.62 ± 2.0)for control and tramadol on day 21st respectively. Concerning, the data of the formalin test group, the same patternwas recorded as hot-plate test group.

As regards, to the withdrawal effect of tramadol, 96-hours withdrawal after the last given tramadol administration (Table 3 & Figure 3), the data obtained on day21st showed significant increase in the latency period of hot-plate tested group (34.63±1.41) after 21consecutive daily intraperitoneal injections of 36mg/kg tramadol compared to control group (8.0±0.76). On contrast, 96 –hours withdrawal after last administration, showed significantly decrease in the latency time (3.38±0.52) compared to the day 21st(34.63±1.41) and control ((8.0±0.76) values.

Formalin test withdrawal group,

The data recorded on day 21st showed significant decrease in the number of licking & biting with mean (51.0 ±2.62)compared to control group (82.75±2.12). In contrast, the 96-hours withdrawal period showed significantly increase in the number of licking &biting (116.88±9.11) compared to control group (82.75± 2.12).

Plantar- incision hyperalgesia evaluation.

As regards heat hyperalgesia (Table 4& Figure 4). Thewithdrawal latencies for heat responses on plantar incision group showed significant decrease ($p \leq 0.05$) with mean (2.88±0.35) compared to control value (7.38±0.52). Concerning tramadol treated plantar incision group, the recorded results showed significant increase ($p \leq 0.05$) in withdrawal latency period (15.38±1.41) compared to both plantar incision group before tramadol administration (2.88±0.35) and control group (7.38±0.52).

Cytokines evaluation

Cytokines production were estimated 2hr- after plantar incision; IL-1, IL-6, and TNF-α.(Table 5 & Figure 5). The present results, showed significant increase in the levels of these cytokines production of plantar incision groups (43.58±2.14, 108.38±8.33, and 505.25±11.67 as compared to control groups (10.65±0.49,41.01±0.86, and105.50±3.89) respectively. Cytokines production after Tramadol-

administration, showed significant decrease in the levels of IL-1(13.90±1.56), IL-6 (41.83±1.72), and TNF-α (163.63±19.41) compared to the level of cytokines in the plantar incision group before tramadol administration (43.58±2.14, 108.38±8.33, and 505.25±11.67) respectively.

Table (1): Effect of acute dose of tramadol 300mg/kg (LD_{50}) of either paw withdrawal latency period (sec)(hot-plate test) and no. of licking & biting (formalin test).

Parameter	Control	Tramadol (300 mg/kg)
Mean paw latency period (sec) (Hot- platetest)	7.25 ± 1.04	37.0 ± 1.85*
Mean no. of licking & biting (Formalin test)	79.50 ± 13.33	25.13 ± 2.70*

*: Statistically significant at $p \leq 0.05$

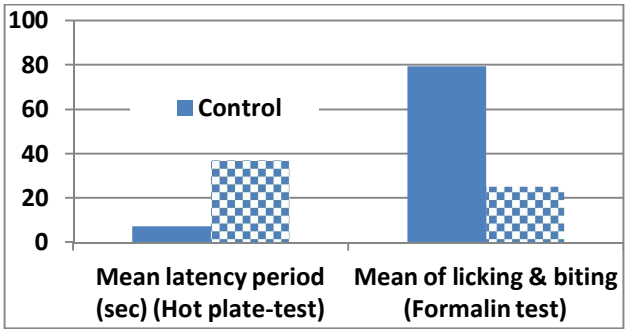

Figure (1): Effect of acute dose of tramadol 300mg/kg (LD_{50}) on either paw withdrawal latency period (sec) and no. of licking & biting.

Table (2): Cumulative effect of increasing tramadol doses of either paw withdrawal latency period (sec) (hot-plate test) and no. of licking & biting (formalin-test)

Parameter	Control	36 mg/ kg, day 7th	75 mg/ kg, day 14th	150 mg/ kg, day 21st
Mean latency period (sec) (Hot- platetest)	7.63 ± 0.52	31.50^{a} ± 1.93*	39.0^{ab} ± 0.93*	22.62^{abc} ± 2.0*
Mean no. of licking & biting. (Formalin test)	91.13 ± 1.55	29.75^{a} ± 1.28*	45.63^{ab} ± 3.34*	51.75^{abc}±1.28*

a: significant with control groupb: significant with 36mg/kgc; significant with 75 mg/kg
*: Statistically significant at $p \leq 0.05$

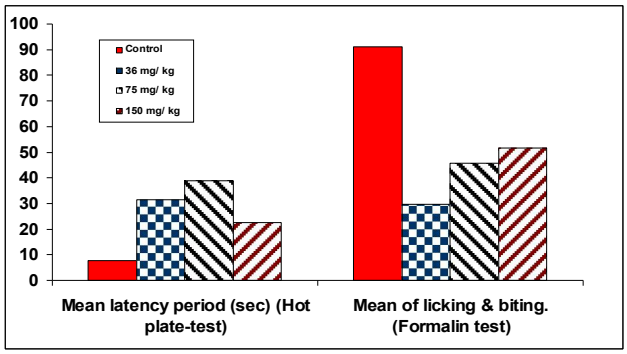

Figure (2): Cumulative effect of increasing tramadol doses 36,75, and 150mg/Kg on either paw latency period (hot-pate test) and no. of licking & biting (formalintest) on days.7, 14, and 21.

Table (3):Effect of tramadol administrations for 21 consecutive days and 96- hr. withdrawal effect on either withdrawal latency period (sec) (hot-pate test) and no. of licking & biting (formalin-test).

Parameter	Control	Day 21	Withdrawal at 96 hours post last dose
Mean latency period (sec) (Hot plate-test)	8.0 ± 0.76	34.63 [a] ±1.41*	3.38 [ab]± 0.52*
Mean of licking & biting. (Formalin test)	82.75 ± 2.12	51.0 [a] ±2.62*	116.88 [ab]± 9.11*

a: significant with control groupb: significant with day21c; significant withdrawal at 96 hours post last dose
*: Statistically significant at $p \leq 0.05$

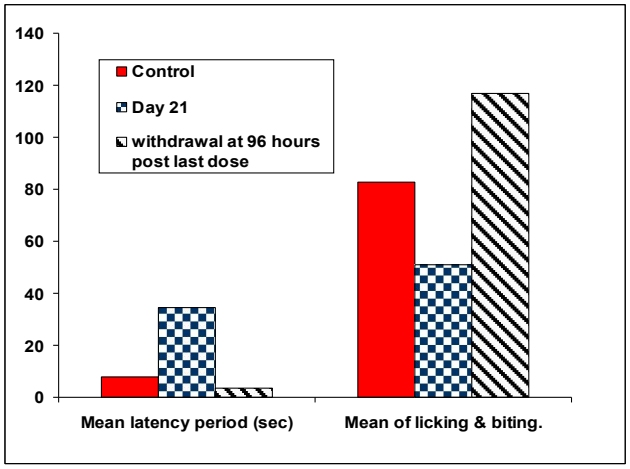

Figure (3): Effect of tramadol administrations for 21 consecutive days and 96- hr. withdrawal effect of either withdrawal latency period (hot-pate test) and no. of licking & biting (formalin-test).

Table (4):Effect of tramadol on thermal hyperalgesia accompanying plantar incision

Parameter	Control	Plantar incision group	Plantar incision + tramadol group
Thermal threshold (sec.)	7.38 ± 0.52	2.88[a] ± 0.35*	15.38[ab] ± 1.41*

a: significant with control groupb: significant with incision group
*: Statistically significant at $p \leq 0.05$

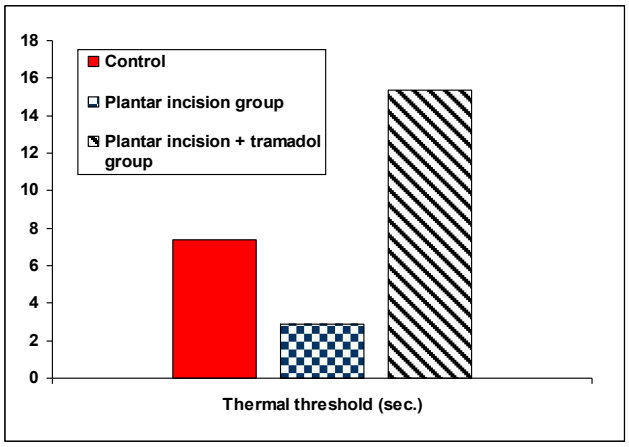

Figure (4): Effect of tramadol on thermal hyperalgesia accompanying plantar incision.

Table (5): Levels of cytokines production in paw plantar incision before and after tramadol treatment

Parameter	Control	Plantar incision (group)	Plantar incision + tramadol (group)
IL-1 pg/ml	10.65 ± 0.49	43.58[a] ± 2.14*	13.90[ab] ± 1.56*
IL-6 pg/ml	41.01 ± 0.86	108.38[a] ± 8.33	41.83[b] ± 1.72*
TNF-α pg/ml	105.50 ± 3.89	505.25[a] ± 11.67	163.63[ab] ± 19.41*

a: significant with control groupb: significant with incision group
*: Statistically significant at $p \leq 0.05$

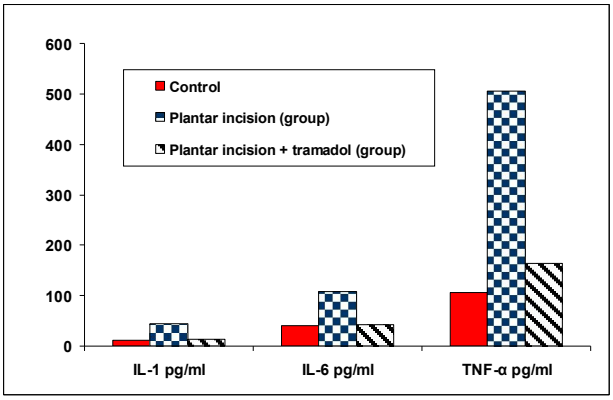

Figure (5): Levels of cytokines production in paw plantar incision before and after tramadol treatment

4.Discussion

In the current study the response of nociceptive thresholds towards tramadol at different dose levels and selected time periods was investigated. Moreover, the present investigation focuses on the immunological changes accompanying tramadol treatment after surgical trauma induced by plantar incisional operation. Also, throws some light on the influence of the withdrawal period on nociceptive threshold in a mammalian experimental model.

Tramadol hydrochloride is a centrally acting analgesic with a partial affinity for the opiate receptor (μ), having analgesic potency estimated to the one tenth that of morphine **(Raphael *et al.*,2000).**

Tramadol has been established as adjunct to non-steroidal anti-inflammatory drugs in the treatment of moderate post-operative pain **(Le Roux and Coetzee, 2000).**

Regarding, the acute single dose of tramadol administration 300 mg/kg (LD_{50}) as evaluation of tramadol antinociceptive activity using hot-plate test. The statistical analysis of the present result, showed significant prolongation in the mean paw withdrawal latency period $p \leq 0.05$. In addition, within tramadol-treated formalin group significant decreases were detected on comparing the number of licking and biting of lesion paw edema with that of control value $p \leq 0.05$. According to **Hara *et al.*(2005) and Berrocoso*et al.*(2007)**, it is known that tramadol binds to mu-opioid receptors with low affinity and inhibits reuptake of mono-amines (serotonin and norepinephrine) in the central nervous system. These are believed to primary contribute to its antinociceptive effects. Another explanation is through the proposed increased turnover of serotonin by the increase serotonin availability to potentiate analgesia then, depletes the neuron **(Atici*et al.*,2004**).

As regards, the effect of cumulative increase doses of tramadol (36, 72, 150 mg/kg body weight), the data recorded over the 7th and 14th days of daily drug administration, showed significant prolongation in the mean withdrawal time among the group of hot-plate test. A significant decreases in the no. of lickingand bitingof paw were recorded as compared to normal value. On the other hand, data recorded on day 21st of daily drug administration, did not induce remarkable changes in the nociceptive threshold in hot-plate animals group and no. of licking and biting paw in formalin animals group as compared to day 7th and day 14th, whereas, significant changes were reported as compared with control value. This can be attributed to the fact that, the long-term use of opioids can however, result tolerance and dependence. There are numbers of studies linking acute receptor desensitization to tolerance and dependence **(Bohn *et al.*,2000; Ueda *et al.*,2001),**receptor desensitization encompasses series of events leading to loss of receptors function. Furthermore, the significant changes of the studied parameters after chronic administration of tramadol in either 36 mg/kg dose level or incremental dose to 72 mg/kg, may be explained through the serotonin type 2c- receptor subtypes that implicated in many important effects of

serotonin including pain. **Ogata *et al.*(2004)** Suggested that tramadol inhibits the serotonin 2c-receptor function and the mechanism of this inhibitory effect seems to involve competitive displacement of the serotonin binding to the 2c-receptor. Another explanation is through the proposed increased turnovers of serotonin to increase serotonin availability to potentiate analgesia, then depletes the neurons.The pathological results of **Atici*et al.*(2004)** confirmed the neuron degenerative effect of the incremental doses of tramadol which probable contributes to the rat cerebral dysfunction. In addition, **Burkey*et al.*(1999)** Provided evidence for an antinociceptive effect of dopamine in the rat cerebral cortex that mediated through descending nociceptive inhibition of spinal neurons suggesting that dopamine acts tonically in the cortex to inhibit nociception.

In addition, along the same line within tramadol – treated withdrawal group for 21 consecutive days and 96-hours withdrawal period, there was significant reduced changes in the mean withdrawal latencies period and increases in the no. of licking and billing of the lesion pawas compared to their values on the day 21st and control group $p \leq 0.05$. It is known that opiate abuse exerts adaptive changes in brain functions, including many aspects of neuro-transmission. The results of the present investigation concerning withdrawal period, showed the excitatory effect in that period through the highly decreased in the mean paw latency time and increased no. of licking and biting. This can be explained in view that, the excitatory effect in that period may be due to the elevation of excitatory amino acids such as glutamic and aspartic acids level which comes in accordance with **Sepulveda *et al.*(2004)** who reported that opiate abuse exerts extensive changes in brain function including excitatory neurotransmission, also could be expression of new adjustments in central nervous system neurotransmission after descentinvasion of the chronic administration of the drug. Moreover, according to **Laviolette*et al.*(2004)** the functional conduction of the rat GABA-A receptor switched from an inhibitory to an excitatory signaling mode due to tolerance and subsequent withdrawal.

Additional explanation of the reduced withdrawal latency period(W.L.P) and increases no. of licking and bites among the rats in 96- hours withdrawal group, may also, explain through the dopaminergic neurons of the chronically treated rats seem to depend on continuous tramadol administration for their normal functioning. This is in line with earlier study, **Attila and Ahtee (1984)** reporting that dopaminergic neurons of the chronically treated rats seem to depend on continuous morphine administration for their normal functioning and confirmed the dysregulation of noradrenergic transmission by morphine. Moreover, earlier studies, also, showed that repeated exposure of rats to morphine and other drugs of abuse induces an increase in the reactivity of nerve terminals during withdrawal, enhancing the exocytotic release of various neurotransmitters, including dopamine **(Heidbreder*et al.*,1996)** andnorepinephrine. These neuro-adaptations are through underline the persistence of drug-induced behavioral sensitization **(Vanderschuren and Kalivas,2000).**

Heat hyperalgesia induced by plantar incision

Heat hyperalgesia is assessed by measuring paw withdrawal latency of rats to heat stimulation,which is applied to the middle of incision using a focused radiant heat source **(Zahn and Brennan, 1999)**. Variety of pharmacological studies have been performed to validate the pain related behaviors induced by plantar incision.. Clinical studies show that extent of muscle rather than skin injury influences the magnitude of pain at rest after surgery**(Ogonda et al.*et al.*,2005; Dorr *et al.*,2007 ; Martinez *et al.*,2007).**

It has been clearly shown that hyperalgesia to thermal stimulation were achieved at maximum severity 2 hours after surgery and lasted for 2 - 4 days before spontaneous remission. The selected time period of 2 hours post-operation was found to be adequate for the assessment of analgesic drug properties on incisional pain **(Bernnan et*et al.*,1996)**. According to **Liu *et al.*(2008)** pain generated by plantar incision in the hind paw in rat model may present a useful model for surgical trauma. In the present study using thermal threshold to plantar incision in rat model, the data recorded for pain threshold before incisional operation, did not show any significant differences between the hind paws among all rats in control group. After surgical procedure, a significant prolongation in the time released to response to the thermal stimulation after tramadol administration ($p \leq 0.05$).

This analgesic effect of tramadol, can be attributed to the activation of both pain inhibiting systems; the opioid and mono-aminergic systems. Also, because compound known to block mono-amine uptake potentially have the antinociceptive effects of opioid including tramadol, the antinociecptive potency and profile of tramadol may derive from its combined opioid binding activity and inhibitions of monoamine uptake **(Sacerdote*et al.*,1997**). In conclusion, tramadol exerts its anaolgesic effect by inhibiting the re-uptake of serotonin and norepinephrine, as well as by weak opioid receptor agonist.

Modulation of cytokines production by tramadol after surgical trauma

Based on the problematic issue, and because the fluctuations in the level of cytokines production are rapid and inconsistent, its difficult to provide a consent explanation for the changes observed here in for each cytokine individually.

Tissue injury or the presence of foreign material initiates a series of pathophysiological events that characteristics of the initiating factor trigger the release of a unique range of pain mediators that control the threshold and activation of nociceptors (Ferreira, 1993). The immune system and different cytokines could be influenced by surgery **(Salo,1992; Henslere*t al.*,1997)** IL-6, along with its pro-inflammatory effects, is a sensitive and early marker of tissue damage**(Nagahiro*et al.*,2001; Raeburn *et al.*,2002).** The recorded data indicated that, IL-1, IL-6 and TNF-α were significantly increased 2 hours post incision and reversed by tramadol. Therefore, it is suggested that some molecules in addition to mu-opioid receptors might be responsible for the antinociceptive effect of tramadol and cytokine production.

Moreover, the antinociceptive effects of tramadol are mediated not only via an opioid mechanism, but also, mainly via a separate non-opioid mechanism, due to a the inhibition of neuronal uptake of nor epinephrine and serotonin **(Kayser*et al.*, 1992; Raffa*et al.*, 1992).** Furthermore, in depressed patients, prolonged treatment with antidepressants normalized the symptoms and reduces the increased serum IL-6 levels **(Hashioka*et al.*,2007).** Therefore, the activation of serotonergic system might be involved in the immune effects induced by the administration of tramadol. The non-opioid mechanism may play a key role in immunomodulation by tramadol.The effects of tramadol on cytokine production may be mainly attributed to intrinsic immunomodulatory properties of tramadol due to the serotonergic descending inhibitory system, SThe decrease of IL-6 production after administration of tramadol, can help to attenuate postoperative immunosuppression **(Sacerdote*et al.*,1997; 1999; 2000; Gaspani*et al.*,2002 and Wang *et al.*,2003).**

In summary, the antinociceptive mechanism of tramadol combined with the opioid and serotonergic system may play an important role in immune responses. Moreover, tramadol was associated with decreased IL-1, IL-6 and TNF-α levels, suggesting that it may suppress the inflammation induced by incision.

Conclusion

Considering analgesic and immunosuppressive effects, tramadol treatment may be a good choice for treatment of acute and chronic pain. It is important to consider tramadol's ability to inhibit serotonin re-uptake when prescripting the drug for patients already taking drugs with serotonergic activity. It is possible that subject stabilized on selective serotonin re-uptake inhibitor SSRIs or other antidepressants could be susceptible to developing serotonin toxicity upon starting tramadol therapy. Also, tramadol dosage should be adjusted according to the severity of pain. Moreover, with the increasing use of tramadol for pain control, it is important for physicians to be aware of its potentially lethal side effects, particularly if consumed or prescripted in appropriately large dose. Although lower as compared to other opioids, tramadol has a potential for physical and psychological dependence. finally, tramadol exerts its analgesic effects through inhibiting the re-uptake of serotonin and norepinephrine and also, by weak opioid receptor agonist.

References

1. Atici S, Cinel L, Doruk N, Aktekin M, Akea A, Camdeviren H, Oral U. (2004): Opioid neurotoxicity: Comparison of morphine and tramadol in an experimental rat model. Int J Neursosci 114 (8):1001-11.
2. Attila LM, Ahtee L. (1984): Retardation of cerebral dopamine turnover after morphine withdrawal and its enhanced acceleration by acute morphine administration in rats. NaunynSchmiedebergs Arch Pharmacol 327: 201-7.
3. Berrocoso E, de Benito MD, Mico JA. (2007): Role of serotonin 5-HT1A and opioid receptors in the antiallodynic effect of tramadol in the chronic constriction injury model of neuropathic pain in rats. Psychopharmacology (Berl) 193(1):97–105.
4. Bohn LM, Gainetdinov RR, Lin FT, Lefkowitz RI, Caron MG. (2000): Mu opioid receptor desensitization by beta –arrestin-2determine morphine tolerance but not dependence. Nature 408: 720-3.
5. Brennan TJ, Vandermeulen EP, Gebhart GF. (1996): Characterization of a rat model of incisional pain. Pain 64(3):493–501.
6. Burkey AR, Carstens E, Jasmin L. (1999): Dopamine reuptake inhibition in the rostral agranular insular cortex produces antionociception. J Neurosci 19(10): 4169-79.
7. Calixto JB, Campos MM, Otuki MF, Santos ARS. (2004): Anti-inflammatory compounds of plant origin, part II. modulationof pro-inflammatory cytokines, chemokines and adhesionmolecules. PlantaMedica 70(2): 93–103.
8. Clark JD, Shi X, Li X, Qiao Y, Liang D, Angst MS, Yeomans DC. (2007): Morphine reduces local

cytokine expression and neutrophil infiltration after incision. Mol Pain 3(1):28.
9. Dorr LD, Maheshwari AV, Long WT, Wan Z, Sirianni LE. (2007): Early pain relief and function after posterior minimally invasive and conventional total hip arthroplasty. A prospective, randomized, blinded study. J Bone Joint Surg Am 89: 1153-60.
10. Faron-Górecka A, Kuśmider M, Inan SY, Siwanowicz J, Dziedzicka-Wasylewska M. (2004):Effects of tramadol an α_2-adrenergic receptors in the rat brain. Brain Research 1016(2): 263-7.
11. Ferrero-Miliani L, Nielsen OH, Andersen PS, Girardin SE, (2007): Chronic inflammation: importance of NOD2 and NALP3 in interleukin-1β generation. Clinical and Experimental Immunology 147(2): 227–35.
12. Filip M, Wydra K, Inan SY, Dziedzicka-Wasylewska M, Drzegalinski E. (2004):Opoid and monoamine system mediate the discriminative stimulus of tramadol in rats. Eur J Pharmacol 498(1-3): 143-51.
13. Gaspani L, Bianchi M, Limiroli E, Panerai AE, Sacerdote P. (2002): The analgesic drug tramadol prevents the effect of surgery on natural killer cell activity and metastatic colonization in rats. J Neuroimmunol 129(1-2):18–24.
14. Gyurkovska V, Alipieva K, Maciuk A. (2011): Anti-inflammatory activity of devil's claw in vitro systems and their active constituents. Food Chemistry 125(1): 171–8.
15. Hara K, Minami K, Sata T. (2005): The effects of tramadol and its metabolite on glycine, gamma-aminobutyric acid A, and N–methyl-D-Aspartate receptors expressed in xenopus oocytes. Anethesia and Analgesia 100(5): 1400-5.
16. Hashioka S, Klegeris A, Monji A, Kato T, Sawada M, McGeer PL, Kanba S. (2007): Antidepressants inhibit interferon-gamma-induced microglial production of IL-6 and nitric oxide. ExpNeurol 206(1):33–42.
17. Heidebreder CA, Thompson AC, Shippenberg TS. (1996): Role of extracellular dopamine in the initiation and long-term expression of behavioral sensitization to cocaine. J Pharm ExpTher (278): 490-502.
18. Hensler T, Hecker H, Heeq K, Heidecke CD, Bartels H, Barthlen W, Wagner H, Siewert JR, Holzmann B. (1997): Distinct mechanisms of immunosuppression as a consequence of major surgery. Infect Immun 65(6): 2283–91.
19. Hunskaar S, Fasmer OB, Hole K. (1985):Formalin test in mice, a useful technique for evaluating mild analgesics. J Neurosci Methods 14: 69-76.
20. Hussein SZ, Yusoff KM, Makpol S, Yusof YAM. (2012):Gelam honey inhibits the production of proinflammatory, mediators NO, PGE2, TNF-α, and IL-6 in carrageenan-induced, acute paw edema in rats. Evidence-Based Complementary and Alternative Medicin 2012: 1-13.
21. Kayser V, Besson JM, Guilbaud G. (1992): Evidence for a noradrenergic component in the antinociceptive effect of the analgesic agent tramadol in an animal model of clinical pain, the arthritic rat. Eur J Pharmacol 224(1):83–8.
22. Laviolette SR, Gallegos RA, Henriksen SJ, van der Kooy D. (2004): Opiate state controls bi-directional reward signaling in the ventral tegmental area via GABAA receptors.Nature Neuroscience7: 160-9.
23. Le Roux PJ, Coetzee JF. (2000):Tramadol today.CurrOpinAnaesthesiol 13(4):457-61.
24. Liu YM, Zhu SM, Wang KR, Feng ZY, Chen QL. (2008): Effect of tramadol on immune responses and nociceptive thresholds in a rat model of incisional pain. J Zhejiang Univ Science B 9(11):895-902.
25. Martinez V, Fletcher D, Bouhassira D, Sessler DI, Chauvin M. (2007): The evolution of primary hyperalgesia in orthopedic surgery: quantitative sensory testing and clinical evaluation before and after total knee arthroplasty. AnesthAnalg 105: 815-21.
26. Matthiesen T, Wohrmann T, Coogan PP, Uragg H. (1998): The experimental toxicology of tramadol : an overview. Toxicology Letters 95: 63-71.
27. McColl G. (2004): Tumour necrosis factor alpha inhibitors for the treatment of adult rheumatoid arthritis. J ExperiCliniPharma 27(2): 43-6.
28. Mueller M, Hobiger S, Jungbauer A. (2010): Anti-inflammatory activity of extracts from fruits, herbs and spices. Food Chemistry 122(4): 987–96.
29. Nagahiro I, Andou A, Aoe M, Sano Y, Date H, Shimizu N.(2001): Pulmonary function, postoperative pain, and serum cytokine level after lobectomy: a comparison of VATS and conventional procedure. Ann ThoracSurg 2001; 72(2):362–5
30. Niemand C, Nimmesgern A, Haan S, Fischer P, Schaper F, Rossaint R, Heinrich PC, Müller-Newen G.(2003): Activation of STAT3 by IL-6 and IL-10 in primary human macrophages is differentially modulated by suppressor of cytokine signaling 3. J Immunol 170(6): 3263–72.
31. Ogata J, Minami K, Uezono Y, Okamoto T, Shiraishi M, Shiraishi M, Shigematsu A, Uela Y. (2004): The inhibitory effects of tramadol on 5-hydroxytryptamine type 2c receptors expressed in xenopus oocytes. AnesthAnalg 98(5): 1401-6.
32. Ogonda L, Wilson R, Archbold P, Lawlor M, Humphreys P, O'Brien S, Beverland D (2005): A minimal-incision technique in total hip arthroplasty does not improve early postoperative outcomes, a prospective, randomized, controlled trial. J Bone Joint Surg Am 87:701-10.
33. Paget GE, Barnes JM. (1964): Interspecies dosage conversion scheme in evaluation of results and quantitative application in different species. In: Laurance DR, Bacharach AL (eds). Evaluation of

drug activities: pharmacometrics. London,New York: Academic press.1:160-2.
34. Pergolizzi JV Jr, van de Laar MA, Langford R, Mellinghoff H, Merchante I M, Nalamachu S, O'Brien J, Perrot S, Raffa RB. (2012):Tramadol/paracetamol fixed-dose combination in the treatment of moderate to severe pain. J Pain Res 5: 327–46.
35. Pothiawala S. (2011): Tramadol overdose: a case report. J ProceSinga Healthcare 20: (3):219-23.
36. Raeburn CD, Sheppard F, Barsness KA, Arya J, Harken AH. (2002): Cytokines for surgeons. Am J Surg 183(3): 268–73.
37. Rafati A, Yasini SM, Dashti-Rahmatabadi MH, Pakdel S, Norani F. (2006): Tramadol dependence rate as compared with morphine in rats. World J Med Sci 1(1): 40-3.
38. Raffa RB, Friderichs E, Reimann W, Shank RP, Codd EE, Vaught JL. (1992): Opioid and nonopioid components independently contribute to the mechanism of action of tramadol, an 'atypical' opioid analgesic. J PharmacolExpTher 260 (1):275–85.
39. Raffa RB. (2001): Pharmacology of oral combination analgesics: rational therapy for pain. J Clin Pharm Ther 2001; 26: 257–64.
40. Raphael J, Leo MD, Rajesh-Narendran MB, Barbara-DeGuiseppe MD. (2000): Methadone detoxification of tramadol dependence. J Substance Abuse Treat 19: 297-9.
41. Sacerdote P, Bianchi M, Gaspani L, Manfredi B, Maucione A, Terno G, Ammatuna M, Panerai AE.(2000): The effects of tramadol and morphine on immune responses and pain after surgery in cancer patients. AnesthAnalg 2000; 90(6): 1411–4.
42. Sacerdote P, Bianchi M, Gaspani L, Panerai AE. (1999): Effects of tramadol and its enantiomers on concanavalin-A-induced proliferation and NK activity of mouse splenocytes: involvement of serotonin. Int J Immunopharmacol 21(11): 727–34.
43. Sacerdote P, Bianchi M, Manfredi B, Panerai AE. (1997): Effects of tramadol on immune responses and nociceptive thresholds in mice. Pain 72(3): 325–30.
44. Salo M. (1992): Effects of anaesthesia and surgery on the immune response. ActaAnaesthesiolScand 36(3): 201–20.
45. Samaka RM, Girgis NF, Shams TM. (2012): Acute toxicity and dependence of tramadol in albino rats: relationship of nestin and notsch 1 as stem cell markers. J Am Sci 8(6): 313-27.
46. Sepulveda J, Oliva P, Contreras E. (2004): Neurochemical changes of the extracellular concentrations of glutamate and aspartate in the nucleus accumbens of rats after chronic administration of morphine. Eur J Pharmacol 483(2-3): 249-58.
47. Sheeran P, Hall GM. (1997): Cytokines in anaesthesia. Br J Anaesth 78(2): 201–19.
48. Sobe PW, Parran TV, Grey SF, Adelman CL, Yu J. (2003) :The use of tramadol for acute heroin withdrawal: a comparison to clonidine. J Addict Di 22(4): 1-4.
49. Takeshita N, Yamaguchi I. (1995):Meta-chlorophenylpiperazine attenuates formalin-induced nociceptive responses through 5-HT1/2receptors in both normal and diabetic mice. Br J Pharmacol 116: 3133-8.
50. Tamaskar R, Parran YV, Heggi A, Brateanu A, Rabb M, Yu j. (2003): Tramadol versus buprenorphine for the treatment of opiate withdrawwl: a retrospective cohort control study. J Addiction Dis 22(4): 5-12.
51. Tayebi P, Kheirkhah F, Tayebi G, Moghadamnia AA. (2008). Tramadol effect on morphine dependency and analgesia in mice.Int J Pharmacol 4(6): 452-9.
52. Ueda H, Inoue M, Mustumoto T.(2001): Protein kinase c-mediated inhibition of mu-opioid receptor internalization and its involvement in the development of acute tolerance to peripheral mu-opioid again analgesia. J Neuro-Sci 21: 2967-73.
53. Vanderschuren LJMJ, Kalivas PW. (2000): Alteration in dopmainergic and glutamatergic transmission in the induction and expression of behavioral sensitization: a critical review of preclinical studies. Psychopharmacol 151: 99-120.
54. Wang W, Danielsson A, Svanberg E, Lundholm K. (2003): Lack of effects by tricyclic antidepressant and serotonin inhibitors on anorexia in MCG 101 tumor-bearing mice with eicosanoid-related cachexia. Nutrition 19(1):47–5.
55. WHO. (2000): Achieving balance in national opioids control policy: guidelines for assessment Geneva.
56. WHO. (2012): WHO's pain ladder [web page on the Internet]. Geneva: Available from: http://www.who.int/cancer/palliative/painladder/en/ [Accessed May 12, 2011].
57. Xiong Q, Tezuka Y, Kaneko T.(2000): Inhibition of nitric oxide by phenylethanoids in activated macrophages European Journal of Pharmacology 400 (1) 137–144
58. Zahn PK, Brennan TJ (1999): Primary and secondary hyperalgesia in a rat model for human postoperative pain. Anesthesiol 90: 863-72.