Global Journal of Current Research Vol. 1 No. 2. 2013. Pp. 70-76

Full Length Research Paper

Effects of Acute Sub-lethal Dose of Tramadol on α_2-Adreergic Receptors and Liver Histopathology in Rat

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Abstract
Tramadol is an atypical opioid with monoamine re-uptake inhibition properties. The aims of the current study were 1) to investigate the effects of acute oral tramadol administration (sub-lethal dose) on the binding of [^{3}H] RX 821002, a selective α_2-adrenergic receptor ligand, in the rat brain,2) and its pathological effect on liver of rat .The results revealed that; Tramadol (single dose), at 3 hr after dosing, induced a significant decrease in the alpha$_2$-adrenergic receptors in all brain regions studied (hippocampus; cerebral cortex; thalamus). Liver histopathology revealed that: Leucocytic sinusoidal permeation, congested blood vessels in the portal tract, degeneration of some hepatocytes and vacuolation of hepatocytes.

Keywords: *Tramadol; α_2-adrenergic receptors, Liver histopathology.*

Introduction

In recent years, it has been postulated that tramadol, used mainly for the treatment of moderate to severe pain, might display a potential as an antidepressant drug**.**

Tramadol is marketed as a racemic mixture of the (1*R*, 2R) - and (1*S*, 2S)-enantiomers with a weak affinity for the μ-opioid receptor (approximately 1/6000th that of morphine; Gutstein & Akil, 2006). The (1*R*, 2R)-(+)-enantiomer is approximately four times more potent than the (1*S*, 2S)-(–)-enantiomer in terms of μ-opioid receptor affinity and 5-HT reuptake, whereas the (1*S*, 2S)-(–)-enantiomer is responsible for noradrenalin reuptake effects (Shipton, 2000). These actions appear to produce a synergistic analgesic effect, with (1*R*, 2R)-(+)-tramadol exhibiting 10-fold higher analgesic activity than (1*S*, 2S)-(–)-tramadol (Goeringer et al., 1997).

Histopathological and biochemical changes due to chronic usage of morphine or tramadol in liver and kidney in rats had confirmed by Atici et al., (2005) where, Serum ALT, AST, LDH, BUN and creatinin levels were significantly higher in morphine group compared to the control group. Serum LDH, BUN and creatinin levels were significantly increased in the morphine group compared to the tramadol group. Light microscopy revealed severe centrolobular congestion and focal necrosis in the liver of morphine and tramadol groups, but perivenular necrosis was present only in the morphine group. The main histopathologic finding was vacuolization in tubular cells in morphine and tramadol groups.

The aims of the current study were 1) to investigate the effects of acute oral tramadol administration (sub-lethal dose) on the binding of [^{3}H] RX 821002, a selective α_2-adrenergic receptor ligand, in the rat brain,2) and its pathological effect on liver of rat .

Materials and Methods

Tramadol

The certified reference standard of Tramadol was provided from October Pharma Co., Egypt, and was of >99 % purity.

Experimental animals

48 male Wistar rats, weighing between 180–200 g were used. As a standard protocol, all the rats were housed in a quiet non-stressful environment for one week before the study. Wistar rats were divided into four main groups.

Animals were raised in the Animals House Unit in Faculty of Veterinary Medicine, Cairo University. They were maintained in plastic cages with stainless steel wire lids; (bedded with wood shavings); on a standard laboratory feed diet. Animals were housed at constant room temperature (20-22 °C), 60% humidity and light cycle of 12h. /day.
All rats were given 45–50 kcal/day normal rat chows during the experimental period.
The animal group (n = 24), divided into three groups received tramadol .Each group consisted of 9 rats (*n*=9) the groups were received tramadol at doses of 40 kg^{-1} (1/10 LD_{50}).
The control group (*n* = 3) were received distilled water. Rats were sacrificed, three of each group or sub-group at constant intervals; 1 hr, 2 hr and 3 hr respectively. The experiments were repeated twice.

Laparotomy and Histopathological examination
Specimens
Midline laparotomy was performed and liver, kidney and brain specimens were obtained.

Histopathological examination
Liver tissue from all animals was collected after the animals were killed and then fixed in buffered 10% formaldehyde PH 7.0 (phosphate puffer). After 48 hr, the fixed tissue was paraffin embedded. Paraffin blocks were cut serially in10–15 6-lm slices and stained with hematoxylin and eosin stains.

Chemical analysis
Determination of Tramadol
Materials
The certified reference standard of Tramadol was provided from October Pharma Co., Egypt, and was of >99 % purity. All organic solvents were of HPLC grade and were purchased from Merck. Sodium carbonate was of analytical grade and purchased from El Naser pharmaceutical Chemical Co., (Egypt).

Preparation of standard solution
Tramadol standard solutions of 100 µg/mL were prepared in methanol and stored at - 20 ºC. Working standard solutions were prepared by dilution of the corresponding stock standard solution with methanol and stored at 4 ºC.
Calibration standard solutions were prepared in solvent (methanol) and at 0.1-10.0 µg/mL concentration range. Matrix matched standard solution were prepared by evaporating to dryness portion of the final extract solutions obtained from control sample and taking up the remained residue with the calibration solution.

Sample preparation
As for the blood sample (0.5 ml), 2 ml of extraction solution (methylene chloride : diethyl ether, 3:2 v/v) and 0.5 ml sodium carbonate solution (0.5 M) were added into 5 ml polyethylene tube, the screw cap was closed and the tube vigorously shaken for 10 min using a vortex at a maximum speed. The extracts were centrifuged for 5 min at 2000 rpm and 4ºC. Then, 1ml of the supernatant was taken, and evaporates to dryness. The residues were redissolved in 1 ml of methanol, filtered through 0.22 µm PTFE filter (Millipore, USA) and transferred into a 1.5 ml glass vial for HPLC-FLD analysis.

Instrumental determination
The HPLC analysis was performed with an Agilent 1260 HPLC system (USA), with quaternary pump, autosampler injector, thermostat compartment for the column and fluorescence detector (FLD). The chromatographic column was Zorbax C_{18} XDB (250 mm x 4.6 mm, 5 µm film thicknesses). The column was kept at room temperature. Flow rate of mobile phase (methanol/water = 40 + 60, v/v) was 1 ml/min., and injection volume was 20 µl. Excitation and emission wavelength for detection of tramadol was set at 200 and 301 nm, respectively. The residues in the real samples were tentatively identified by comparing the retention times (RTs) of the sample peaks with the RTs of the injected standards.

Linearity, recovery and detection limits
To determine the linearity a 100 µg/mL stock solution was prepared and working solutions (0.1, 0.5, 1, 5, 10 µg/mL) were prepared by diluting the stock solution with methanol. A standard calibration curve of tramadol was constructed by plotting analyte concentrations against peak areas (Fig. A). The results showed good linearity with a correlation coefficient of R^2 =0.9999.

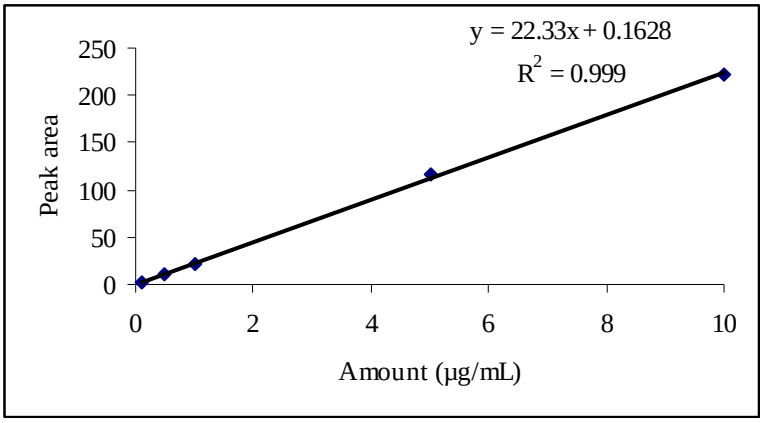


Figure A. Linearty correlations for tramadol calibration curve

The LOD (limit of detection) of the test compound was 0.01 µg/mL and resulted by considering a signal-to-noise ratio of 3 with reference to the background noise obtained for the blank sample. The limit of quantification (LOQ) of the method was 0.05 µg/mL, the S/N of which was generally > 10. The matrix effect of the present method was investigated by comparing standards in solvent with matrix-matched standards for 5 replicates at 0.1 µg/mL. The results showed that, no interfering endogenous peak appeared, and the retention times of the tested analytes at the spiked sample completely matched those of the standard samples. The analytes were eluted as separate symmetric peaks.

Recoveries of tramadol at different fortification levels, i.e., 0.05, 0.1, 0.5 and 1.0 µg/mL, were determined in five replicates to validate and evaluate the accuracy of the method. The obtained recoveries were in the acceptable range 90.1-95.4%. The coefficient variation of the methods (CV %) for repeatability ranged from 5.3 % to 11.5 %, within acceptable range. It is suggested that tramadol can be detected with good precision

Determination of α_2-adrenergic receptor ligand

The rats used for biochemical experiments were sacrificed at 1, 2 and 3 hr after a single dose (acute treatment) of tramadol. Brains used for in situ hybridization were rapidly dissected and frozen by immersion into cold heptanes in a dry ice bath and stored at -70 jC until sectioned (Faron-Go´reckaet al., 2004)**.** Consecutive coronal sections (12 Am) were cut at _19 jC using a cryostat Jung CM3000 (Leica). The identification and nomenclature of brain structures were based on the rat brain atlas of Paxinos and Watson (1998).

Autoradiographic analysis of a_2-adrenergic receptors

Receptor binding with [^{3}H] RX 821002 (specific activity: 41 Ci/mmol; Amersham Pharmacia Biotech, UK) was visualized using the procedure described by Holmberg et al. (2003). Briefly, the sections were first preincubated for 10 min at room temperature in 50 mM phosphate buffer, pH7.4, and then incubated for 20 min at room temperature in a buffer containing 0.5 nM [3H]RX 821002. Nonspecific binding was determined by incubation of parallel sections in the presence of 5 AM rauwolscine hydrochloride (Tocris, Ellisville, MO, USA). Following the incubation period, tissue sections were washed twice in ice-cold phosphate buffer and once in distilled water, and then dried with cool air. Radiolabelled dried tissue sections were apposed to tritium-sensitive screens (FujiImaging plate) along with [^{3}H] microscales (Amersham Pharmacia Biotech) and the images were obtained with the use of FujiFilm BAS 5000 Phosphoimager. The autoradiograms were analyzed using a computer imaging system and quantified with the use of computer-generated curves derived from the standards. The images of sections showing nonspecific binding were subtracted from the images of adjacent sections with total binding. Data are expressed as the mean FS.E.M. (fmol/ mg tissue) from seven animals per group.

Results and Discussion

The plasma concentration time profile of tramadol is shown in Fig. 1. The half-life of tramadol obtained in this study was 3.2 h**.**

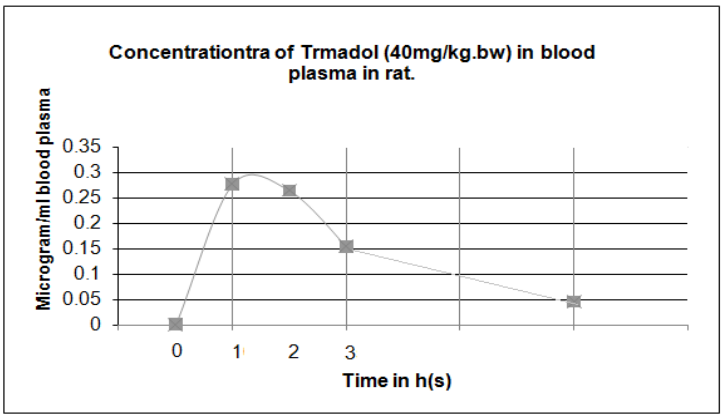
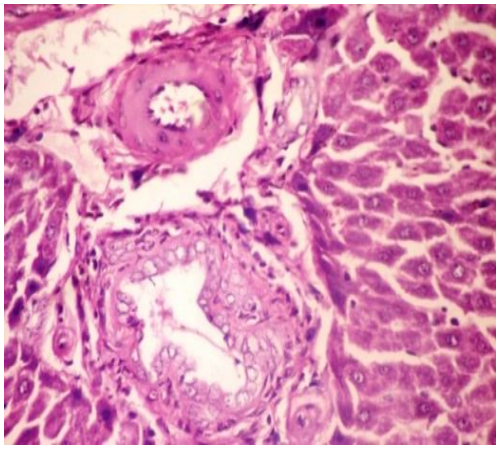


Fig. (1): Mean ± SD plasma tramadol (h) concentrations following oral administration of tramadol (40 mg kg^{-1}) body weight to healthy rats.

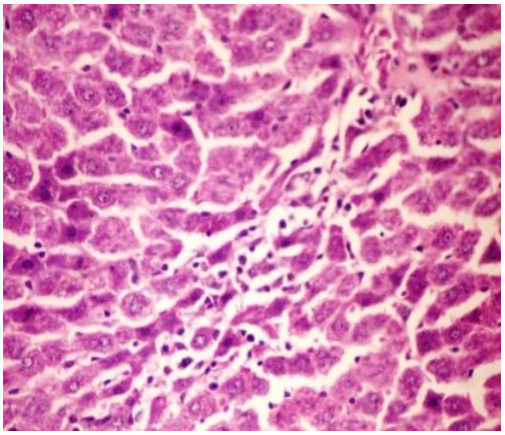
Liver histopathology

Light microscopy of the findings were revealed leucocytic sinusoidal permeation, congested blood vessels in the portal tract, degeneration of some hepatocytes and vacuolation of hepatocytes.

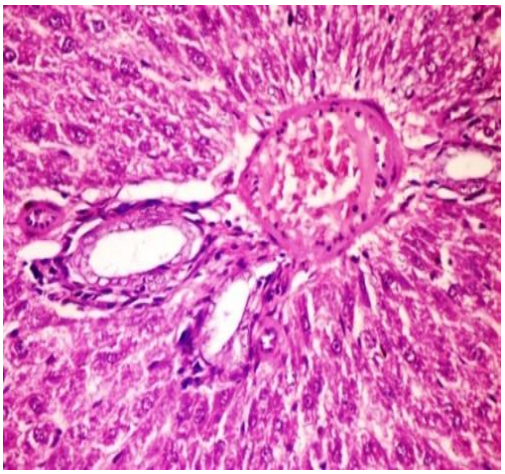
Liver is responsible for the metabolism and excretion of tramadol (Coughtrie *et al* 1989; Milne *et al* 1997). Morphine may cause hepatotoxicity and nephrotoxicity during its metabolism (Van der Laan *et al.,* 1995). Incubation of adult human hepatocytes with opioids, in therapeutic doses, for 24 h, is unlikely to produce irreversible damage to these cells in chemically defined culture conditions (Gomez–Lechon *et al* 1988). Experimental studies have also supported toxic effects of chronic use of opioids on liver and kidneys. Borzelleca *et al* (1994) reported increased levels of ALT, AST and LDH in rats after long-term usage of morphine like agent levo-alpha–acetylmethadol (LAAM) HCL. Centrolobular hypertrophy in the liver was also reported in the same study.
Every drug has been associated with hepatotoxicity almost certainly due to the pivotal role of the liver in drug metabolism. Hepatic metabolismis, first and foremost, a mechanism that converts drugs and other compounds into products that are more easily excreted and that usually have a lower pharmacologic activity than the portent compound (Poppers 1980; Tolman1998). A metabolite may have higher activity and/or greater toxicity than the original drug.



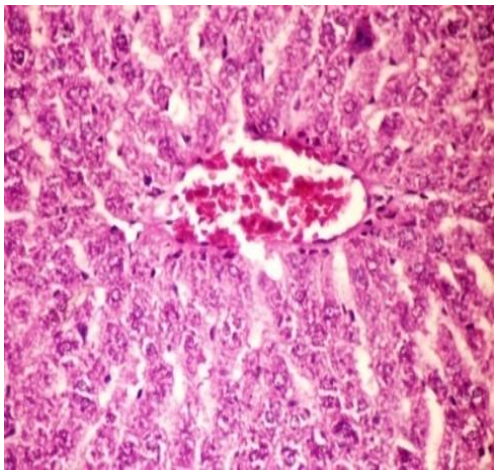
1hr post-dosing: hyperplasic bile duct and lymphocytic permeation (H&E X 400)



2hr post-dosing: Liver showing leucocytic sinusoidal with disorganized hepatic cord (H&E X **400).**



3hr post-dosing: Liver showing congested blood vessel n the portal tract (H&E X 400).



24 hr post-dosing: Liver showing congestion with degeneration of some hepatocytes (H&E X 400).

Fig. (2): Light microscopy of the histopathologicla findings in rat liver**.**

Table (1) : Histopathologic findings in liver.

	Tramadol 40 mg/kg (*n* = 12)	Control (*n* = 12)
Liver		
Hydropic degeneration &disorganization	2/12	12/12*
Hemorrhages	1/12	12/12
Congestion	2/12	11/12
Sinusoidal dilatation	4/12	11/12
Heptocytes vacuolation	2/12	12/12

**Histopathological findings were normal except minimal congestion in two individuals of the control group. Histopathologic changes of injury are summarized in Table, 1.*

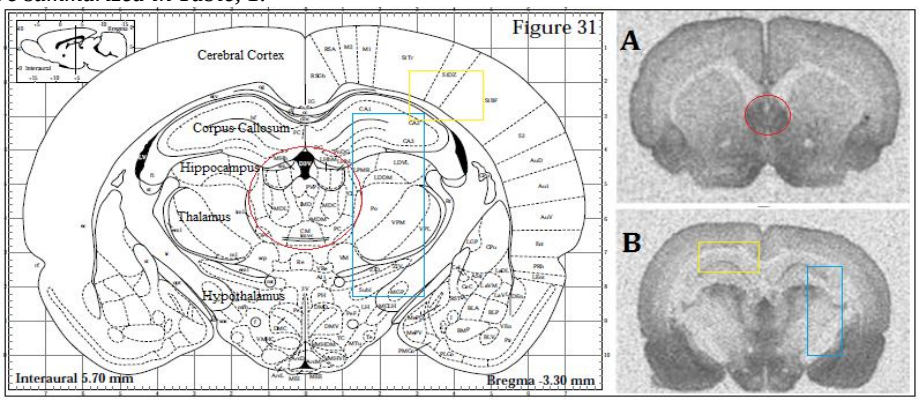


Fig. (3): Binding binding of [^{3}H] RX 821002, a selective α_2-adrenergic receptor ligand in the rat brain. The maps of A–C are adapted from a standard rat brain atlas (Paxinos and Watson, 1998) indicating the levels that sections were cut. [^{3}H] RX 821002 binding sites in the rat brain (cortex; hippocampus; thalamus).

Tramadol receptors

The present study investigated the effects of acute tramadol administration on the binding of [^{3}H] RX 821002, a selective alpha$_2$-adrenergic receptor ligand, in the rat brain.

Tramadol (single dose), at 3 hr after dosing, induced a significant decrease in the alpha$_2$-adrenergic receptors in all brain regions studied (hippocampus; cerebral cortex; thalamus).

Treatment with tramadol 40 mg kg^1, oral gavages, once induced statistically significant down regulation of [^{3}H] RX 821002 binding sites in the rat brain.

Brain α_2-adrenergic receptors (alpha$_2$-ARs) have been implicated in the regulation of anxiety, which is associated with stress. Environmental treatments during neonatal development could modulate the level of brain alpha$_2$-AR expression and alter anxiety in adults, suggesting possible involvement of these receptors in early-life programming of anxiety state (Gobbi *et al.*, 2002).
Tramadol is a very weak μ-opioid receptor agonist, induces serotonin release, and inhibits the reuptake of norepinephrine (Reimann and Schneider, 1998; Gobbi *et al.*, 2002). Tramadol is converted to *O*-desmethyltramadol, a significantly more potent μ-opioid agonist. The opioid agonistic effect of tramadol and its major metabolite(s) is almost exclusively mediated by such μ-opioid receptors. This further distinguishes tramadol from opioids in general (including morphine), which do not possess tramadol's degree of receptor subtype selectivity and which are much stronger opiate-receptor agonists. Similarly, the habituating properties of tramadol (such as they are) are arguably mainly due to μ-opioid agonism with contributions from serotonergic and noradrenergic effects.

The analgesic action of tramadol is not fully understood, but it is believed to work through modulation of serotonin and norepinephrine in addition to its relatively weak μ-opioid receptor agonism.

Faron-Go´recka et al., (2004) in recent years, it has been postulated that tramadol, used mainly for the treatment of moderate to severe pain, might display a potential as an antidepressant drug. They investigated the effects of acute and repeated tramadol administration on the binding of [^{3}H]RX821002, a selective a2-adrenergic receptor ligand, in the rat brain. , tramadol (20 mg/kg, i.p.) administered acutely (single dose), at 24 h after dosing, induced a significant decrease in the a2-adrenergic receptors in all brain regions studied.

The central noradrenergic system is implicated in the etiology of depression by the known therapeutic action of antidepressant drugs, ascribed to their inhibitory action at presynaptic transporters of serotonin and noradrenaline. In connection with the serotonin hypothesis, α_2-adrenergic receptor downregulation has been revitalized (Mongeau et al., 1994), since the downregulation of a2-adrenergic heteroreceptors controlling serotonin release may be an important factor in antidepressant action. Indeed, growing evidence suggests dysfunction of α_2-adrenergic receptors in depression (Katona,et al.,1997).

Table (2) : Effects of acute treatment with Tramadol (TRAM) on the binding of [^{3}H] RX 821002 to **α_2**-adrenergic receptors in the rat brain (3 hours post treatment).

Brain region	Control	Tramadol (40mg kg^{-1} BW)
Hippocampus formation	61.40±2.63	56.50 ±2.15*
Cortex	128.46±3.24	122.84±3.23*
Thalamus	131.72±3.55	125.44±4.68*

Fmol/mg tissue); *: Significant (P< 0.05).

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