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A Novel Controlled Release Subcutaneous Implant of Tramadol Hydrochloride: In Vivo Bioavailability and Neurochemical Study

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Abstract

The current study investigated the pharmacokinetic profile of a newly developed biodegradable subcutaneous tramadol implant and shed some light on neurochemical changes. Ribbons of polycaprolactone polymer were loaded with tramadol HCl in two quantities (T350, T650) and implanted in back skin of rats. Plasma tramadol levels were monitored for 45 days and pharmacokinetic parameters were evaluated in addition to analgesic activity. Cortical oxidative stress indicators, orexin, and serotonin levels were determined. The CB-1 receptor and PPAR-α receptor protein levels were assayed. Both T350 and T650 loaded ribbons attained a sustained release profile for 45 days. T650 implants achieved higher bioavailability than T350. However, the analgesic efficacy of tramadol started once it was implanted and decreased along time indicating accelerated tolerance. Oxidative biomarkers were of normal range. The ventrolateral periaqueductal gray (vlPAG) analgesic pathway involving orexin, endocannabinoid, and serotonin was found to be affected in relation to developing tolerance. Induced cortical 5-HT and orexin-A and decreased level of DA after implanting T350 and T650 were observed. PPAR-α and CB-1R were of elevated abundance in cortex after T650 implants.However, pharmacodynamics reported a sensitizing effect related to vlPAG pathway. The implant achieved long stable release profile but of diminished analgesic activity.

Keywords: Tramadol; implant; controlled release; polycaprolactone; analgesic; opioid; pain management

1. Introduction

Since 1977, Tramadol was promoted in Germany as a powerful analgesic alternative to opioids. It was first made available to the public in the United States and worldwide in 1995 [1]. It is used all around the world for the management of moderate to severe pain from health problems such as joint disease, cancer discomfort, and disc prolapse [2]. The proper consumption of opioids for chronic pain remains a significant worldwide health concern due to related opioid use disorders observed in 20 % of patients prescribed opioids [3]. Tramadol acts by activating μ-opioid receptors (MOR) and inhibiting the reuptake of norepinephrine and serotonin (5-HT) [4]. After being metabolized, tramadol convertsto o-desmethyltramadol, a strong opioid agonist with a higher affinity for the MOR and higher relative intrinsic efficacy than the parent drug [5, 6]. Currently, oral route is the most prevalent type of treatment with tramadol. Following a single oral dose, tramadol is completely absorbed with 70% bioavailability owing to first pass hepatic metabolism with a relatively slow onset of action more than one hour and a peak effect between 2 to 4 h [2]. The intravenous route produces potent analgesia and shorter onset of action but, invasive and necessitates medical supervision [7]. Tramadol is converted to active metabolites by the CYP2D6 enzyme. The extreme phenotypes, which account for 5–10% of the Caucasian population, are linked to poor metabolizers' inability to respond well to pain medication or a higher risk of adverse drug reactions in ultra-rapid metabolizers. A greater percentage of women are affected by these impacts [8]. Tramadol by the oral route can have some abuse liability though less than prototypic MOR

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agonists, while parenteral route bypasses the first-pass metabolism thus minimize this side effect. Tramadol usually exerts less negative effects compared to traditional opioids, like morphine and fentanyl [9]. Tramadol side effects include sleeplessness, convulsions, nausea, and vomiting [10]. Despite these benefits, tramadol has a short half-life and requires continuous infusion or repeated dosages to produce a sustained analgesic effect [11]. Moreover, it had a high probability of treatment program discontinuation when taken orally at daily dosages (600 mg) because of its adverse effects [12]. Commercial formulations of prolonged release of tramadol, Zytram XL®, are readily approved for oral administration in the USA and Canada. When comparing the maximum amount of a 200 mg tablet Zytram XL® to an oral solution Tridural® (available in Canada) containing 100 mg of Tramadol, the pharmacokinetic profile of the former reveals a 34% reduction in max. It is feasible to observe a steep initial slope in both cases, which is followed by a prolonged release phase and a greater bioavailability [13]. Other examples are injectable polyhydroxybutyrate microspheres and adhesives based on chitosan and carbohydrate hydrogels for skin delivery [14]. There is an urgent need for developing a dosing regimen tailored to each patient's needs and provide around-the-clock analgesia, thus minimizing frequent administration and providing acceptable tolerability. Moreover, may reduce adverse effects related to concentration such as dizziness, nausea or vomiting. The pharmaceutical industry has been significantly impacted by the development of Drug Delivery Systems (DDSs) due to their ability to maintain a specific drug concentration in blood plasma, ideally within the therapeutic index, and to deliver a predetermined amount of therapeutic substance to the site of action for an extended period of time [15]. It is the most effective way to improve the drug's bioavailability, minimize side effects, and lower dosing frequency while also improving its therapeutic impact [16]. A variety of natural and synthetic polymers have been utilized in this context owing to their low toxicity, enhanced bioavailability, biocompatibility, biodegradability, and chemical feasibility [17]. Implantable biodegradable DDS resemble an effective alternative for traditional dosage forms. Natural and synthetic biodegradable materials break down in vivo into safe, biocompatible byproducts that can be removed by regular metabolic processes. The principal advantages of the sustained and controlled release formulations depend on their capacity to sustain the effective therapeutic times by controlling drug concentration in blood [18]. In a previous study, we developed and evaluated a biodegradable subcutaneous implant with potential for substantially extended release for more than 45 days [19]. The present investigation studied and compared the efficacy of two concentrations of tramadol loaded ribbons implant (T350 and T650) by evaluating: (1) the pharmacokinetic profile; (2) analgesic activity by hot plate testing and (3) neurochemical changes underlying antinociceptive pathway.

2. Material and Methods

2.1. Drugs and chemicals

Tramadol hydrochloride was generously supplied by Sigma pharmaceuticals, Egypt. poly (Ɛ-caprolactone) (PCL, MW 80,000 g/mol), Dichloromethane (DCM), PVA (Mw =67,000), β-cyclodextrin (BCD) (Wacker, Burghausen were purchased from Sigma–Aldrich (Germany). All solvents were HPLC-grade and supplied from Sigma Co., USA.

2.2. Preparation of polycaprolactone-tramadol ribbons

The biodegradable polymeric polycaprolactone (PCL) ribbons (5x15 mm) were prepared and loaded by tramadol with slip casting solvent evaporation technique as previously detailed [19]. Briefly, 350 and 650 mg of tramadol hydrochloride were dispersed in the prepared PCL solution (2.5% w/v). The resulting mixes were then cast onto 5x15mm ribbon-shaped molds, and they were let to dry overnight at room temperature. For every rat in the experimental groups, a 75 mm^2 ribbon was used as an implant from each concentration batch.

2.3. Animals

Female Wistar rats (200-230 g) were supplied from The Animal House colony of NRC. The animals were housed in individual cages and kept on a 12-hour light/dark cycle. Lights were on from 7:00 to 19:00 every day, and they had unrestricted access to food and drink. ARRIVE guidelines were maintained. In compliance with the NIH publication (No. 8023, revised 1978), the NRC's ethical committee granted ethical approval (#16-330). A total of 40 rats were assigned to pharmacokinetic experiment as 5 rats/time point with maximum of two withdrawal episodes in a week to avoid any distress or change in blood volume. Rats were divided into three main groups and assigned for tramadol injection (50 mg/kg, once, s.c.) or T350 implant or T650 implant. Each group was divided into 5 rat subgroups for different time points blood withdrawal. Other 6 rats were kept as control rats.

2.4. Subcutaneous implantation

To implant the prepared ribbons, rats were anesthetized with inhalation (1% isoflurane) in an anaesthetization chamber. Every rat had its back shaved and disinfected by betadine. Incisions were made through the skin and a $1cm^2$ subcutaneous pouch was created by blunt dissection. Ribbons were implanted and stabilized into the pouch then the skin incisions were closed and sanitized.

2.5. Pharmacokinetic study

After implantation or tramadol s.c. injection, blood samples were taken at 0, 0.5, 1, 2, 4, 6, 12, 24, 48, 72, 96, 120, 144, 240 hours, 14, 21, 37, and 45 days from different sets that each rat was sampled 3 times only at >6 hours interval. Plasma was separated and stored at -80°C for analysis of drug level. For ethical purposes and to avoid blood volume disturbances, each rat was subjected to no more than three 48 hour separated blood sampling. Five replicates were made for each time point. Plasma Tramadol-time profiles were analyzed using the following pharmacokinetic parameters: $t_{1/2}$, t_{max}, C_{max}, AUC0-∞, and MRT.PK Solver (version 2), an EXCEL add-in program, was used to determine these parameters using non-compartmental approach.

2.6. Pharmacodynamic study

Hot plate test was employed to test analgesic activity of the implants. Rats were tested on 6 h, 24 h, 7 d, and 14 d on a hot plate instrument (UgoBasil, USA) at 55.0±1.0°C. Each rat was placed and the time to first lift or lick its forepaw was recorded with a cut off set to 30 sec to prevent injury. The same observer conducted each experiment. The information was presented as a percentage of the maximum effect (MPE%= test latency time–baseline/cut-off–baseline×100).

2.7. Determination of tramadol HCl

Plasma was extracted by alkaline liquid-liquid extraction method. Plasma samples were centrifuged for 10 min at 3000 rpm at ambient temperature. NaOH (0.1 M) was used to alkalinize the samples to a pH of about 11. Five milliliters of a solvent mixture (ethyl acetate: hexane, 1:4 v/v) were used to extract tramadol from plasma. The samples were centrifuged for 30 minutes at 4000 rpm and 4 °C after being vortexed. The supernatants were gathered and allowed to evaporate in nitrogen at 35 °C. The mobile phase was used to reconstitute the dried residues. The RP-18 column was used for the separation, and the mobile phase consisted of 30% acetonitrile with 0.1% triethylamine and 70% phosphate buffer (0.01 M, pH 5.9), at a flow rate of 0.75 mL/min. Tramadol was detected at λ = 218 nm. An R2 score of 0.99 indicated that a straight line fit the calibration data.

2.8. Study of cortical molecular changes

At the end of 45 days, brains were excised; cortices were separated and instantly kept at -80°C for analysis. Dopamine and serotonin were determined by means of HPLC (Agilent 1100 series, Waldbronn, Germany) measurement in methanolic 10% cortex homogenate. ELISA measurement of orexin (Fine test, Wuhan fine biotech Co., China), CB-1R and PPAR-α R (Sunbiotech, China) in cortical homogenates were performed following the manufacturer's instructions. Oxidative stress of brain cortex tissue was assayed by determining tissue levels of GSH according to the colorimetric method of Ellman, 1959 and MDA according to the method of [20].

2.9. Statistical Analysis

Results are expressed as the mean ± standard error. One way ANOVA followed by Tukey's multiple comparison was used to test of significance. Statistical significance was considered at $p<0.05$.

3. Results

3.1. Pharmacokinetic study of tramadol loaded polycaprolactone ribbons

Pharmacokinetic analysis revealed that s.c. injection of tramadol (50 mg/kg) resulted in rapid and sharp increase in plasma tramadol post-dose level with C_{max} of 60.04 µg/ml at t_{max} of 4 h and decreased rapidly with elimination half-life of 38.95 h and

could be detected in plasma up to 144 h (6 days), while after s.c. implantation with T350 and T650, levels of tramadol increased rapidly with C_{max} of 90.9 and 83.8 µg/ml, respectively (Figure 1).

Although C_{max} was comparable between T350 and T650, there was a marked difference in t_{max} (72 hrvs 378 h) and the subsequent distribution and elimination phases. Following implantation with T350, plasma tramadol levels decreased rapidly after reaching C_{max} with elimination half-life of 1909 h (79.5 days) and elimination rate constant of 0.0003745 h-1. Then, concentrations of tramadol decreased more slowly providing less fluctuating levels (Table 1).

In contrast, implantation with T650 provided sustained plasma concentrations of tramadol without obvious fluctuations. Steady release of tramadol was observed over about 5 weeks resulting in approx. constant plasma levels. Implantation with T650 provided higher bioavailability in terms of significantly higher AUC and MRT compared to T350 (6 and 5-fold, respectively). Elimination rate constant decreased 20 %. In addition, the lower values of CL observed confirmed relatively higher AUC (Table 1).

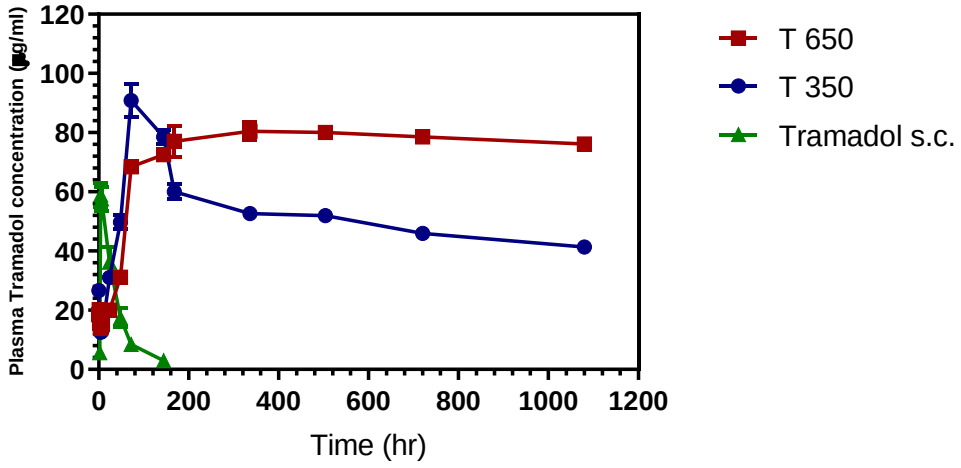

Figure (1): Pharmacokinetic data of T350 and T650 ribbon in rat

Table 1: Pharmacokinetic measures for tramadol-loaded PCL ribbons

	C_{max} (µg/ml)	**t_{max} (h)**	**$t_{1/2}$ (h)**	**λz (1/h)**	**AUC (µg/ml*h)**	**MRT (h)**	**CL/F (mg/µg/ml/h)**	**V_d/F (mg/µg/ml)**
T 350	90.89* ± 5.668	72 ± 0.0	1909 ± 250.4	0.0003745 ± 0.000044	169500 ± 15669	2744 ± 340	0.002098 ± 0.0001816	5.65 ± 0.21
T 650	83.84* ± 1.45	378*@± 42	9525*@ ± 1789	0.0000816*@ ± 0.0000164	1137971*@ ± 198773	13801*@ ± 2580	0.00063*@ ± 0.000118	7.792*@ ± 0.124
Tramadol (50 mg/kg, s.c.)	60.04 ± 4.376	4.667 ± 0.6667	38.95 ± 7.030	0.019 ± 0.00368	2568 ± 68.59	47.65 ± 5.669	0.0195 ± 0.00052	1.100 ± 0.217

*significantly different from Tramadol s.c. group at $p< 0.05$.

@significantly different from T350 group at $p< 0.05$.

3.2. Pharmacodynamic study of tramadol loaded polycaprolactone ribbons

Tramadol implant resulted in delayed pain sensation that lasted more than 24 hours for both T350 and T650. It was observed that the implants caused sensitized pain sensation after 48 hours where the time taken by rat to withdraw its paw decreased by two folds relative to normal rats. The subcutaneous injection of tramadol induced analgesia for the first 24 hours and rapidly declined to normal levels (Figure 2).

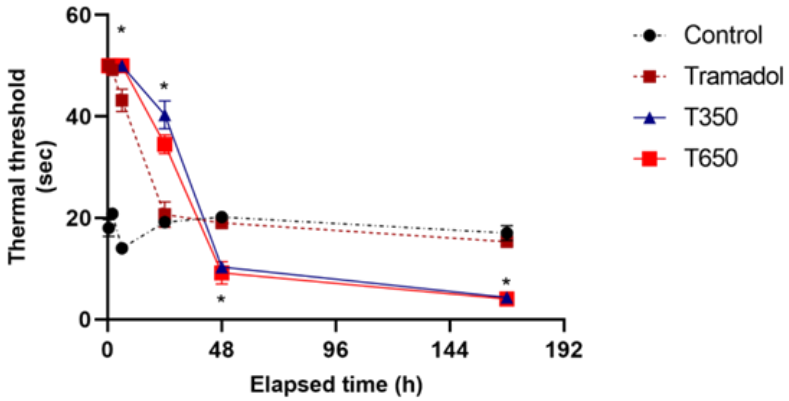

Fig.2: Pharmacodynamic profile of tramadol implant

Results are expressed as mean of n=6±SEM. One-way ANOVA followed by Tukey's test of significance at $p<0.05$ was used.

* against normal control.

3.3. Cortical Oxidative state evaluation

Reduced glutathione (GSH) content of cortical tissue was found to be unchanged after implanting either T350 or T650 ($p>0.05$). Subcutaneous tramadol injection decreased its content significantly compared to normal control ($p<0.05$). Lipid peroxidation was not affected after the implantation ($p>0.05$) but was significantly higher in tramadol injected rats ($p<0.05$). Results are shown in figure (3).

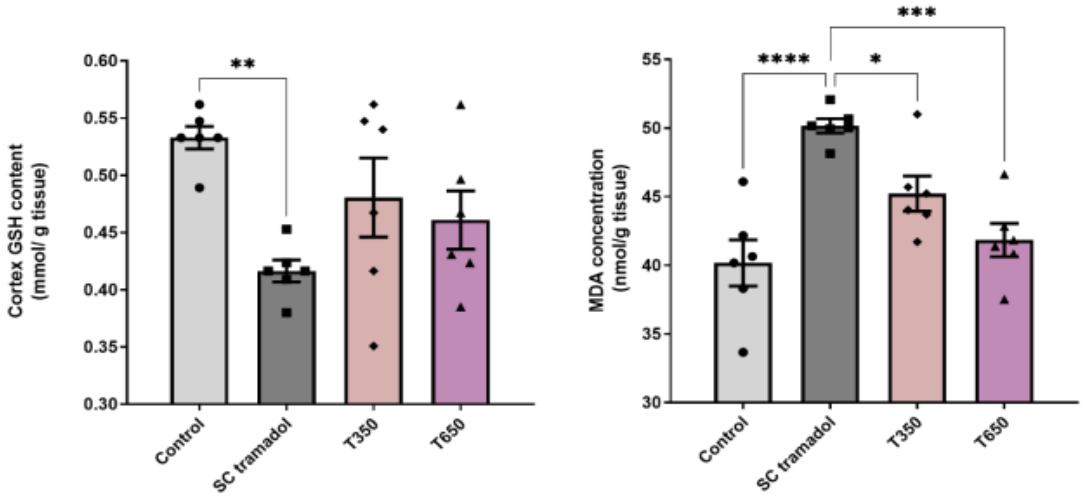

Fig.3: Oxidative stress evaluation of cortical tissue in rats after implantation of tramadol implants

Results are expressed as mean of n=6±SEM. One-way ANOVA followed by Tukey's test of significance at $p<0.05$ was used.

* against normal control.

3.4. Neurochemical state evaluation

Examining the vlPAGantinociception involved proteins revealed that orexin-A was excessively released in cortical tissue ($p>0.05$) along with increased expression of CB-1R following s.c. implantation of T350 and T650. The PPAR-α receptors were at same level as control animals ($p>0.05$). Assay of dopamine and serotonin showed elevated levels of serotonin in cortex tissue of the brain ($P<0.05$) dose dependently, while dopamine level was reduced in a dose dependent manner ($p<0.05$) (Fig.4).

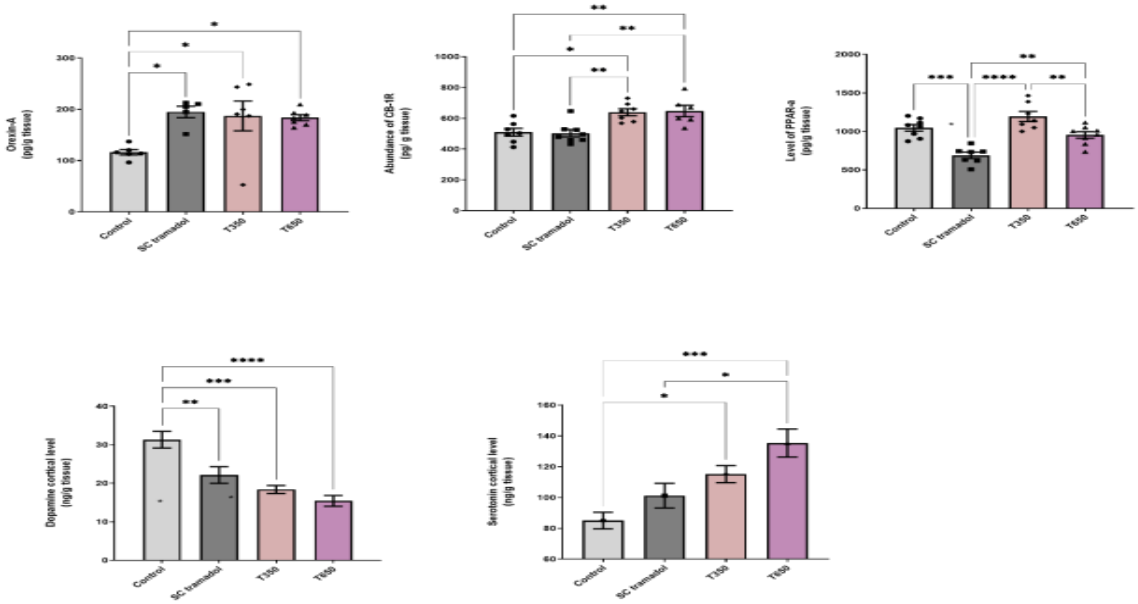

Fig.4: Cortical levels of Orexin-A, CB-1R, PPAR-α, DA and 5-HT in rats after implantation of tramadol implants
Results are expressed as mean of n=6±SEM. One-way ANOVA followed by Tukey's test of significance at $p<0.05$ was used.
* against normal control.

4. Discussion

Preclinical pharmacokinetics and pharmacodynamics of the newly developed tramadol subcutaneous implant is a mandatory procedure, since the in vitro findings sometimes cannot be replicated during in vivo investigations. The current formulations were previously studied in vitro in our lab and showed distinguished efficacy and release profile with good chemical stability and integrity [19]. Application as an analgesic patch in normal rats was evaluated in the current study. It was shown that T650 attained a steady release of tramadol at a therapeutic blood level for 45 days without initial spike; maintained the drug's sustained effective plasma concentrations while reducing peak and trough fluctuations. The use of biodegradable polycaprolactone based ribbons minimized impact of scar tissue as the implants were well-tolerated after subcutaneous implantations without observing any signs of adverse reactions at the implant site. Because of its extended breakdown period and inexpensive cost, polycaprolactone is favored over other polymers and is a potential option for formulations including long-acting administration [21].

Previous in vitro studies presented different preparations that were developed to extend tramadol release but they did not reach the 45 days limit as in the current study, which is mainly attributed to the biodegradable polymer component [22, 23]. The permitted intravenous doses of tramadol are 50 mg and 100 mg injected slowly every 4 to 6 hours. It is estimated that

after IV tramadol regimen, a total tramadol dosage of 350 mg will be used in 24 hours and 650 mg will be loaded over 48 hours, while after oral tramadol regimen, 400 mg will be administered in 24 hours and 800 mg will be administered over 48 hours in total. The present study used the 350 mg and 650 mg doses as a starting dosing load in the skin implants. Bioavailability of tramadol is multiplicated by many folds if administered through other routes than the oral route. Previous studies described more than 5 fold increase in tramadol bioavailability when administered intranasally to rats compared to oral administration [24, 25].

Papini et al. [26] designed a new formulation for tramadol that produced high and constant plasma concentration for more than 180 minutes and resulted in reducing its clearance. In our study, the same sharp initial slope, then a sustained release phase and an enhanced bioavailability was detected for 45 days. Tramadol was also formulated on nanovesicular carrier and delivered into the nasal mucosa layers to achieve more than 60% of maximum effect and extended its mean residence time by 2.5 folds compared to parenteral administration [27].

The skin implant applied in the present study increased the bioavailability of tramadol by more than 50 folds compared to a single subcutaneous injection of same tramadol load. Indeed, blood level of tramadol increased two folds after consecutive multiple doses administration credited to the fact that the metabolizing enzymes are saturated leading to bioavailability enhancement [28]. This can explain the improved AUC after implanting a batch of same limited amount of tramadol but of continuous slowed release in the present investigation rather than single injection.

Usually, efficacy of opioids is measured in terms of antinociceptive activity. The developed tramadol implant should also ensure adequate activity for a prolonged duration of time, thereby minimizing the frequency of consumption of short- acting pain killer medications. However, in the current investigation tramadol induced analgesia for 48 hours then a tolerance to the analgesic effect was developed and even allodynia was observed.

The antinociceptive activity of tramadol is produced through both opioid and nonopioid mechanisms involving μ-opioid receptors activation and inhibiting reuptake of serotonin and norepinephrine [4]. Opioid tolerance is described in several studies where tolerance to morphine was developed after 24 hours of using morphine pump in young rats [29]. Additionally, opioid-induced hypersensitivity is observed with the prolonged use of strong analgesic [30]. Repeated parenteral administration of tramadol did not produce tolerance [31]. Sustained administration of a maximum analgesic dose of tramadol produced adaptation similar to low dose of morphine [32]. However, it is agreed that tramadol is of low physical dependence liability [33].

It is shown that the tramadol implant resulted in elevated cortical level of orexin and increased content of CB1R in brain cortex. Dopamine level was reduced while serotonin level was increased in cortical area. The higher centers in brain cortex are responsible for pain stimulus processing to perceive it as painful [34]. Regulation of nociception occurs via extended projections to different supraspinal parts of brain such as hypothalamus, ventral tegmental area (VTA), locus coeruleus (LC), nucleus accumbens (NAc), hippocampus, periaqueductal gray (PAG), rostral ventromedial medulla (RVM), and dorsal horn (DH) [35].

These structures provide a mechanism through which cortical and subcortical sites process nociception especially in chronic pain situation. PAG receive inputs and send stimulus to LC and RVM, their response is related to 5-HT and noradrenaline release. 5-HT can facilitate the anti-or pro-nociceptive effects depending on the activated receptor either 5-HT7 or 5-HT3 receptors [1]. 5-HT is related to nociceptive transmission in RVM [36]. It is documented that orexin neurons are also involved in pain perception and its neurons are widely distributed in these structures (Fig.5). Moreover, endogenous orexin is involved in μ-receptor desensitization induced in LC [37].

Orexin-A induces antinociception through activation of orexinergic receptors in VTA, which activates the dopaminergic inputs to the NAc [39, 40]. The ventrolateral PAG (vlPAG) matter of the midbrain is an important site for modulating pain [37, 40, 41]. An orexinergic analgesic mechanism in the vlPAG is mediated by 2-arachidonoylglycerol release (2-AG), which acts in in a retrograde manner on CB1R at the presynaptic membrane, inhibiting GABA release and activating the descending vlPAG excitatory neurons [38, 42].

Furthermore, serotonergic role is prominent in the orexin analgesic mechanism, where serotonin modulates the feedback inhibitory mechanism with orexin reciprocally. The RVM includes the serotonergic nucleus raphe magnus as well. The role of

orexin is also extended to the development of tolerance. It was shown that Orexin-1R antagonists in the LC block the analgesic effects of microinjected endorphins and are involved in acceleration of development of morphine tolerance [43].

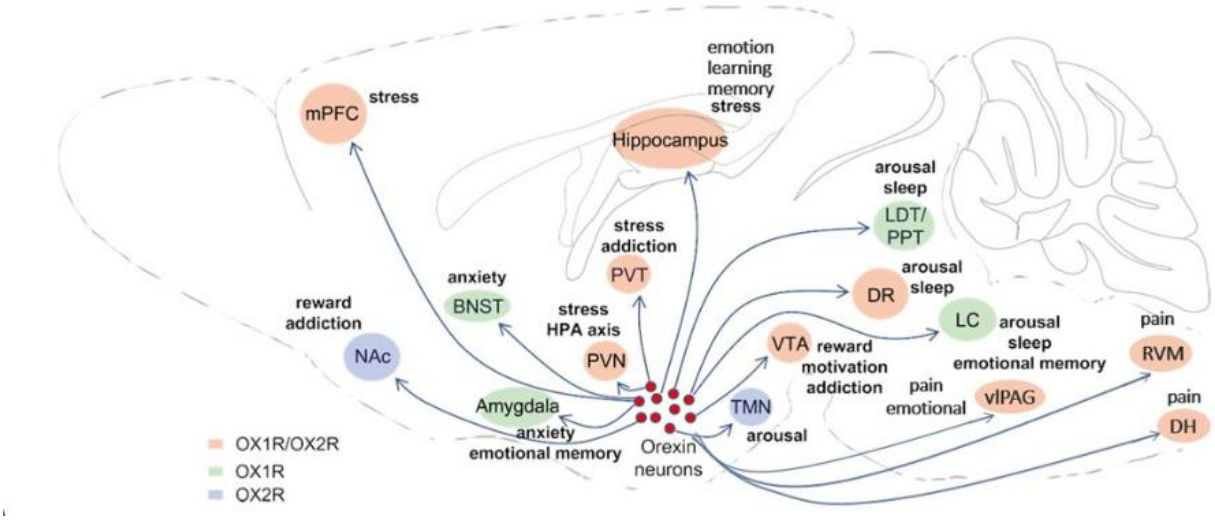

Fig.5: Distribution of orexin neurons in brain (adapted from [38])

It is suggested that tramadol induced the opioid pathway of analgesia and stimulated endorphin release that resulted in opioid tolerance and receptor desensitization conveying increased expression of orexin-A and CB1R in prefrontal cortex. Moreover, the induced sensitization of pain threshold can be related to the serotonergic RVM modulatory nucleus which is implicated in tolerance development and inhibiting the orexinergic analgesic pathway. In addition, CB1R activation is critical for opioid receptor desensitization and internalization. Activation of vlPAG is specifically related to development of central sensitization and results in hyperalgesia and allodynia [36, 44]. PPAR isoforms are expressed in brain regions involved in pain such as amygdala, PAG, and PFC [45, 46]. PPARα activation is related to increased DA signaling and was reported to have anti-nociceptive role [45]. Blocking the receptor exacerbated pain sensation, while PPARα knockout mice were of enhanced noxious sensitivity [47–49]. The present study showed increased expression in relation to prolonged analgesic administration in comparison to single injection suggesting that it is activated only in chronic pain sensation. Therefore, the currently produced sensitization is suggested to be related to the hyperactivity of orexinergic neurons affecting the descending vlPAG pathway implied by accumulated level in brain and its related neurotransmitters represented in dopamine and serotonin. Reactive oxygen species induced oxidative stress is a common pathology associated with multiple disorders and can be referred to as an indicator of safety. GSH is an essential component of the natural defense system, while MDA level is the most common indicator for lipid peroxidation in biomedical sciences [50]. Oxidative protective mechanisms were normally functioning in brain of implanted rats supporting its safety on body tissues. Tramadol oral administration had no oxidative damaging evidence in brains or blood of mice after short term use, while induced a damage after prolonged use [51]. Besides, chronic tramadol treatment was linked to inflammatory and apoptotic changes [52, 53].

5. Conclusions

Taken all together, it can be concluded that tramadol could be successfully formulated into a very long-acting formulation with stable pharmacokinetic profile. However, its application should be for other medical benefits than analgesia since

tolerance is rapidly developed. The opioid induced hyperalgesia is described for the first time after tramadol sustained use and a possible neurochemical change underlying its development was highlighted in the present investigation.

6. Conflicts of interest

There are no conflicts to declare.

7. Formatting of funding sources

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