

Original Article

Effects of systemically administered tramadol hydrochloride on random pattern skin flap survival in rats

 Nadide Ors Yildirim¹,  Elcin Kadan²,  Alperen Kutay Yildirim³,  Okan Ali Aksoy⁴,
 Berk Alp Goksel⁴,  Gokhan Ozkan⁵,  Mehmet Burak Eskin⁵,  Vedat Yildirim⁵

¹Sincan Training and Research Hospital, Department of Anaesthesiology and Reanimation, Ankara, Türkiye

²Health Science University, Gülhane Training and Research Hospital, Department of Pathology, Ankara, Türkiye

³Gazi University Hospital, Department of Cardiovascular Surgery, Ankara, Türkiye

⁴University of Health Sciences, Gülhane Institute of Health Sciences, Department of Animal Experiment and Research Center, Ankara, Türkiye

⁵Health Science University, Gülhane Training and Research Hospital, Department of Anaesthesiology and Reanimation, Ankara, Türkiye

Received: November 28, 2023 Accepted: January 08, 2024 Published online: January 30, 2024

Content of this journal is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License



Abstract

Aim: Tramadol is a weak centrally acting opioid analgesic with μ -opioid receptor agonist effects. It has been explored for its analgesic efficacy through various in-vitro and in-vivo studies. The skin, as the body's largest organ and the protector barrier, is vulnerable to injuries and wounds that can cause significant challenges due to pain, economic burdens and psychological implications. Successful healing of wound involves complex processes and it is influenced by factors such as angiogenesis and nitric oxide levels. This study investigates the effects of tramadol on wound healing in experimental ischemic wound models in rats.

Material and Methods: Two 6 mm ischemic wound models were created on the backs of 30 male Wistar Albino rats, with wound areas cut along the long edges and a sterile silicone material placed between the panniculus carnosus fascia and paraspinosus muscle. Rats were randomized into Tramadol, Control, and Sham groups. The wounds were imaged using a "SONY ILCE-7" camera on days 0, 3, 6, 10, and 14. Wound areas and healing percentages were calculated from the images and recorded for statistical purposes. After 14 days, the animals were sacrificed under general anesthesia for histopathological examination of tissue samples. CD31 and VEGF antibodies were used to evaluate the density and morphology of vascular structures.

Results: Tramadol administration accelerated the healing of wound surface area. Significant differences were found between groups in terms of inflammation, and data recorded in CD34, CD31, and VEGF-stained preparations.

Conclusion: The study found that tramadol positively contributes to wound healing in the acute phase of ischemic wounds by affecting various processes.

Keywords: Flap, inflammation, ischemic wound, tramadol hydrochloride, wound healing

INTRODUCTION

Tramadol hydrochloride ($C_{16}H_{25}NO_2$) is a weak centrally acting opioid analgesic that acts through selective μ receptors. In addition to its weak μ -opioid receptor agonist effect, it inhibits the presynaptic reuptake of noradrenaline (NA) and serotonin (5-HT7), and stimulates the release of 5-HT7 [1]. Its analgesic efficacy has been demonstrated by various in vitro and in vivo

studies [2,3].

Tramadol hydrochloride is metabolized by the hepatic CYP2D6 isoenzyme to O-Desmethyltramadol (M_1), which has greater affinity for μ -opioid receptors than its parent compound, and this metabolite is mainly responsible for opioid analgesic effect [4]. It is known that M_1 metabolite has an analgesic effect approximately 6 times stronger than original preparation and

CITATION

Ors Yildirim N, Kadan E, Yildirim AK, Aksoy OA, Goksel BA, Ozkan G, et al. Effects of systemically administered tramadol hydrochloride on random pattern skin flap survival in rats. Turk J Vasc Surg. 2024;33(1):17-25.



Corresponding Author: Nadide Ors Yildirim, Sincan Training and Research Hospital, Department of Anaesthesiology and Reanimation, Ankara, Türkiye
Email: orsnadide@gmail.com

binds to μ -opioid receptor 200 times more strongly; therefore, it is considered the most pharmacologically active metabolite [5].

The skin, which is the largest organ in the human body in terms of surface area and the first protective barrier, can be exposed to various attacks from time to time. A wound is defined as a deterioration in the integrity of the skin together with the structure and function of the underlying tissue. Injuries are an inevitable part of life and often heal in a smooth process. However, in patients whose healing process is interrupted for various reasons, infection due to wounds is associated with increased mortality and significant morbidity due to pain, limitation in daily activities, and psychosocial consequences. This situation is becoming an increasing burden on the country's economy as well as the psychosocial stress it creates for patients and their relatives. The cost associated with wound treatment in the UK was estimated to be between £4.5 billion and £5 billion [6]. For our country, chronic wounds and related problems are a subject that needs improvement due to both the decrease in the quality of life of the patients and the burden they create on the country's economy.

Wound healing involves four complex but systemically progressing steps that cannot be separated from each other with sharp boundaries [7]. Separate parts of a wound can be at different healing stages at any given time. During angiogenesis, various cytokines and growth factors play an important role in many steps. Treatments aiming to increase the nitric oxide (NO) level in scar tissue may have positive effects on wound healing. Opioids are known to stimulate NO production in tissues [8]. Therefore, opioids may have an enhancing effect over wound healing.

Animal studies investigating the effects of opioid analgesics on wound healing in the literature were mostly performed with superficial applications of these drugs instead of systemic applications [9,10]. In studies with tramadol hydrochloride, drug was used locally instead of systemic use in order to use the local anesthetic effect of the drug [11,12]. In our study, we aimed to examine the contribution of systemically applied tramadol hydrochloride to wound healing in terms of the early phase of proliferation including endothelial proliferation and revascularization.

MATERIAL AND METHODS

This study was supported by the Health Sciences University Scientific Research Projects Coordinatorship with project number 2022/139. Following the approval of the Ethics Committee with the number ETİK 2022/02 on January 27, 2022, from the Health Sciences University Gülhane Animal Experiments Local Ethics Committee, the animals were obtained from the Health Sciences University R&D Center Gulhane Experimental Animals Production and Research Unit. The study was also carried out at the center.

Thirty male Wistar Albino rats over 400 grams were included in our study, and each of them was housed individually in a standard cage specially designed for rats at 21–24°C and 50% air humidity throughout the study. Based on other studies that used similar models with different pharmacological agents, a G-Power analysis was conducted [9,11,12]. The analysis calculated that a minimum of $n=8$ animals per group is required for a study of moderate effect size. Taking into account potential losses during the study, the number of animals in each group was increased by 20% to $n=10$. All rats were randomly divided into three groups involving 10 rats each. Groups were determined as Control (Group C), Sham (Group S), and Tramadol (Group T).

Rats were weighed individually before anesthesia, and their average weight was calculated as 533 grams (min.420-max.620). General anesthesia was achieved by administering a mixture of 40-90 mg/kg Ketamine and 5-10 mg/kg Xylazine intraperitoneally. After the depth of anesthesia was evaluated, the dorsal region of the rats was shaved using the No.40 tip of the Oster A5 brand razor. Rats were fixed with tape from 5 points in the prone position, and their backs were drawn with a pre-prepared standard 10 cm x 3 cm template using a permanent marker.

The wound models were determined as 6 mm punch biopsy areas in the midline inside a template of 10 cm x 3 cm, at a distance of 6 mm from both the outer edges and each other. (Figure 1A).

Following the drawing, asepsis was achieved with 10% povidone iodine. With the help of sterile, disposable 6 mm punch biopsy instruments, tissue pieces were taken from the previously determined points with a depth not exceeding the fascia of the panniculus carnosus muscle, and two full-layer 6 mm wound models were created (Figure 1B).

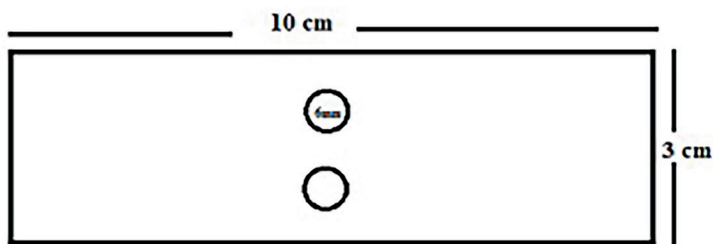


Figure 1. Wound model template on the left side, two 6 mm diameter wound models showed on the right side of the figure

To create the bipedicular flap model, parallel incisions were created with a scalpel in accordance with the template. The fascia of the panniculus carnosus muscle and the paraspinosus muscle below it was dissected (Figure 2A). Sterile silicone material (Figure 2B, black arrow) prepared to create the ischemic tissue

model was placed between the fascia of the panniculus carnosus and the paraspinosus muscle (Figure 2B). The flap was fixed by suturing the incisions and sterile adhesives were adhered to cover the wound areas and suture lines in order to prevent infection of the wound (Figure 2C).

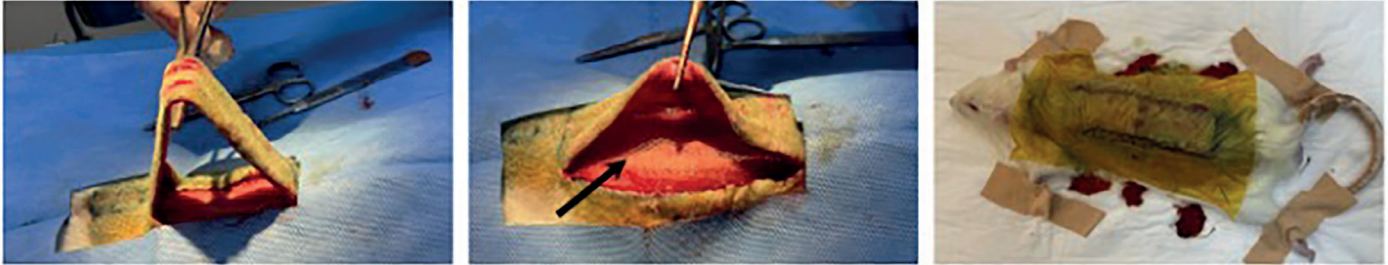


Figure 2. Bipedicular flap model on the left, placement of sterile silicone material showed with black arrow at the middle of the figure, completed surgical procedure showed on the right side of the figure

On the first day of the study, three rats died due to surgical stress within the initial 12 hours post-operation. As a result, 27 remaining rats were re-randomized, with nine rats allocated to each group. To mitigate potential bias, a numbering system was implemented for the rats, and the allocation of injectors to specific groups was determined in advance. The drugs were then prepared in equal milliliter amounts using identical injectors by an assistant who had no contact with the animals. This approach ensured that the practitioner conducting the study would not know which rat belonged to which drug group, thus maintaining the study's objectivity, and minimizing potential sources of bias.

During the study, every 12 hours, a dose of 5 mg/kg tramadol was administered to the Group T in accordance with the Guidelines for Anesthesia and Analgesia in Rats [13]. The Group S of study involved sodium acetate, the solvent of tramadol hydrochloride

(8.3 mg in 100 mg). Sodium Acetate was given at a dose of 0.42 mg/kg twice a day throughout the study. Finally, the Group C received 1 ml/kg of sterile water for the duration of the study.

Except for the unfortunate loss of three rats within the initial 12 hours post-surgery, no further rats experienced mortality during the 14-day follow-up period. Wound healing progress was meticulously monitored in each group throughout the entire 14-day study period. Daily assessments were conducted at a consistent focal length and with identical camera settings, maintaining a standard distance of 60 cm from the subjects. Photographs were taken on the operation day (day 0), as well as on the 3rd, 6th, 10th, and 15th days of the study, using a camera model with the designation "SONY ILCE-7" (Figure 3). A wound area calculation was made using the "ImageJ-Wound Healing Tool" program, and the results were recorded.

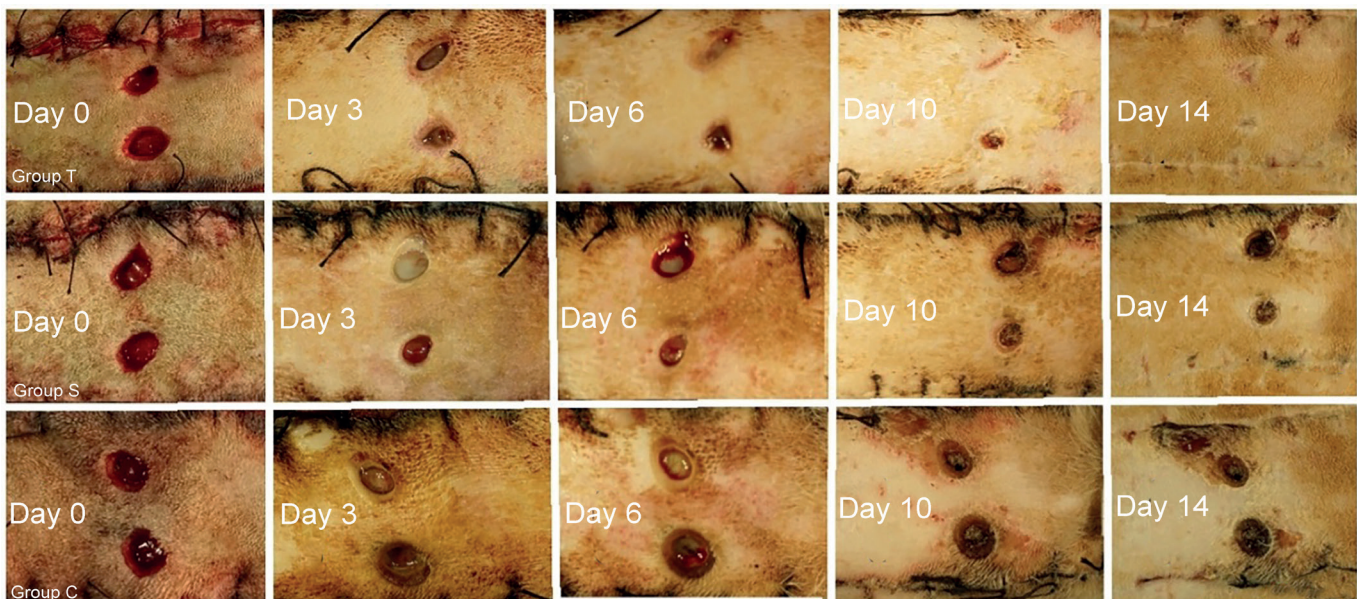


Figure 3. Macroscopic Images of Sample Follow-up of 14-Day Wound Healing of the Groups, The first, 2nd and, 3rd rows represents Group T, Group S and Group C respectively

The wound area measurement calculated on day 0 was recorded as (A0), and other measurements taken were recorded as An (n=number of days). The wound healing percentage by days was calculated using the formula “ $[(A_0 - A_n) / A_0] \times 100$ ” [14]. The percentage changes in wound area were statistically evaluated.

After the predetermined 14-day recovery period was completed, the animals were sacrificed by cervical dislocation under general anesthesia. For the histopathological examination of the tissue from the wound healing area, a fixed area was determined for each subject, and 2 x 2 cm samples were taken. Sections of 5 µm thickness were taken from the paraffin blocks obtained after tissue follow-up and first examined by the hematoxylin & eosin (HE) routine staining method. In order to better evaluate the density and morphology of vascular structures immunohistochemically, CD31, CD34 and VEGF antibodies were added. In favor of better evaluation of the macrophage density in the granulation tissue to be formed, sections with CD68 antibodies were also added. Masson's Trichrome stain was applied to evaluate the collagen amount of the formed connective tissue more accurately. The obtained dyed sections were compared with each other in terms of numerical and qualitative evaluations with light microscopy.

Parametric tests were used without the normality test due to the compatibility of the Central Limit Theorem [15]. In the analysis

of the data, the mean, standard deviation (SD) and n (%) values are given while performing the continuous data statistics. The relationship between categorical variables was evaluated with the Chi-Square Exact test. The Repeated ANOVA test was used to compare the means of more than two dependent measurements, and the One-Way ANOVA test was used to compare the means of continuous measurements in more than two independent groups. In cases of differences, pairwise comparisons were made with Tukey statistics. The statistical significance level of the data was taken as $p < 0.05$. "IBM SPSS 25 version and MedCalc" statistical package program was used to evaluate the data.

RESULTS

Macroscopic Findings

There was no statistically significant difference in the wound area calculations of Group T, Group S, and Group C on the 0th day, but a statistically significant difference was observed in the measurements taken on the 3rd, 6th, and 14th days. Difference in the wound area between groups at a specific time was analyzed by One-Way ANOVA test. In order to measure the change of wound area over time The Repeated ANOVA test was used. In cases of differences, pairwise comparisons were made with Tukey statistics. Measurement results and p-values are shown in Table 1.

Table 1. Change in right and left sided wound areas by time

Wound area of groups (mm ²)	Day 0 $\bar{x} \pm SD$	Day 3 $\bar{x} \pm SD$	Day 6 $\bar{x} \pm SD$	Day 10 $\bar{x} \pm SD$	Day 14 $\bar{x} \pm SD$	p-values
Group T (Right sided)	18.11±1.14	9.91±2.53	6.93±3.16	4.25±2.91	2.13±1.33	<0.001*
Group S (Right sided)	18.01±1.38	16.01±2.47	8.31±2.36	3.55±1.41	0.61±0.42	<0.001*
Group C (Right sided)	18.55±0.58	16.67±1.75	10.28±0.91	5.72±0.94	2.45±0.81	<0.001*
p-values (Right sided wound areas)	0.55**	<0.001**	0.02**	0.08**	0.03**	
p-values (Right sided wound areas)						
Group T-Group S		<0.001***	0.44 ***		0.09 ***	
Group T-Group C		<0.001***	0.02 ***		0.88 ***	
Group S-Group C		0.81***	0.2 ***		0.03 ***	
Group T (Left sided)	18.42±1.31	11.24±2.86	6.44±2.37	3.24±2.64	1.11±0.98	<0.001*
Group S (Left sided)	18.64±3.21	18.47±3.07	10.94±1.85	6.63±1.66	1.99±1.51	<0.001*
Group C (Left sided)	18.83±2.51	19.35±3.52	13.69±1.31	10.38±0.91	3.53±1.06	<0.001*
p-values (Left sided wound areas)	0.94**	<0.001**	<0.001**	<0.001**	0.002**	
Group T-Group S		<0.001***	<0.001***	<0.001***	0.33***	
Group T-Group C		<0.001***	<0.001***	0.002***	0.002***	
Group S-Group C		0.83***	0.01***	0.001***	0.05***	

Data are shown as mean±standard deviation. The different superscripts over p-values (*, **, ***) indicates the utilized statistic test respectively Repeated ANOVA test, One Way ANOVA test, Post Hoc Tukey. Statistically significant p-values are marked in bold font

The closure rates of the calculated wound areas according to the days were calculated with the previously mentioned formula, and the results were evaluated statistically. Wound area changes (%) and p-values for both sides are shown in Table 2.

Table 2. Changes in the areas of wounds on both the right and left sides in terms of percentage over time

Wound area change (%)	Day 0-3 $\bar{x} \pm SD$	Day 0-6 $\bar{x} \pm SD$	Day 0-10 $\bar{x} \pm SD$	Day 0-14 $\bar{x} \pm SD$	p-values
Group T (Right sided)	34.64 $\pm$ 9.75	50.36 $\pm$ 19.42	70.18 $\pm$ 17.37	85.11 $\pm$ 14.74	<0.001*
Group S (Right sided)	11.52 $\pm$ 7.71	53.23 $\pm$ 11.29	80.24 $\pm$ 5.83	96.58 $\pm$ 2.9	<0.001*
Group C (Right sided)	10.25 $\pm$ 7.51	46.16 $\pm$ 4.28	70.06 $\pm$ 4.64	87.24 $\pm$ 3.76	<0.001*
p-values (Right sided wound area change)	<0.001**	0.53**	0.1**	0.03**	
p-values					
Group T-Group S	<0.001***			0.03***	
Group T-Group C	<0.001***			0.87***	
Group S-Group C	0.94***			0.09***	
Group T (Left sided)	39.56 $\pm$ 12.05	55.02 $\pm$ 19.46	78.23 $\pm$ 19.04	93.21 $\pm$ 5.95	<0.001*
Group S (Left sided)	13.78 $\pm$ 6.81	39.18 $\pm$ 14.49	62.8 $\pm$ 12.82	87.85 $\pm$ 11.69	<0.001*
Group C (Left sided)	3.62 $\pm$ 2.25	34.79 $\pm$ 5.09	50.14 $\pm$ 7.35	82.81 $\pm$ 5.87	<0.001*
p-values (Left sided wound area change)	<0.001**	0.02**	0.001**	0.04**	
p-values					
Group T-Group S	<0.001***	0.07***	0.07***	0.37***	
Group T-Group C	<0.001***	0.02***	0.001***	0.04***	
Group S-Group C	0.07***	0.79***	0.15***	0.41***	

Data are shown as mean $\pm$ standard deviation. The different superscripts over p-values (*, **, ***) indicates the utilized statistic test respectively Repeated ANOVA test, One Way ANOVA test, Post Hoc Tukey. Statistically significant p-values are marked in bold font

Histopathological Findings

To objectively evaluate vascular density, we conducted comparisons based on the count of vascular structures within a 1 mm² area. When assessing individual markers, One-Way ANOVA test was used and statistically significant differences were observed: between Group T and Group C in CD34 stained preparations, among Group T, Group S, and Group C in CD31 stained preparations, and between Group T and Group S in VEGF stained preparations. (Figure 4A and Figure 4B)

Granulation tissue and macrophage density developed in the scar tissue were examined with CD68 antibody-applied sections. High-power field (HPF) was used to allow counting in a narrower area with the lens. When the results obtained were evaluated statistically with One-Way ANOVA test, no significant difference was observed between the groups. The statistical results and p-values of the examinations made with immunohistochemical markers are shown in Table 3.

Fibrosis density was investigated by Masson's Trichrome staining method, considering the thickness of the connective tissue

collagen fibers and the staining intensity. Chi-Square Exact test was utilized in order to examine the fibrosis density. There was no significant relationship between the groups (Figure 4C).

Epidermis thickness was measured in μ m with NIS-Elements D application in HE sections. When the measurement results were evaluated, a significant difference was found between the groups. Although there was no statistically significant difference between Group T and Group C in terms of epidermis thickness, it was observed that the epidermis thickness was significantly thinner in Group S.

Granulation tissue thickness and tissue thickness were measured using a grid in HE sections under the light microscope. Statistical studies were carried out both separately and by proportioning the measurements obtained. When the statistical data were evaluated with One-Way ANOVA test, it was seen that there was no significant difference between the groups. Statistical data and p-values obtained by measuring epidermis thickness, subcutaneous fat tissue thickness, granulation tissue thickness, and tissue thickness are shown in Table 4.

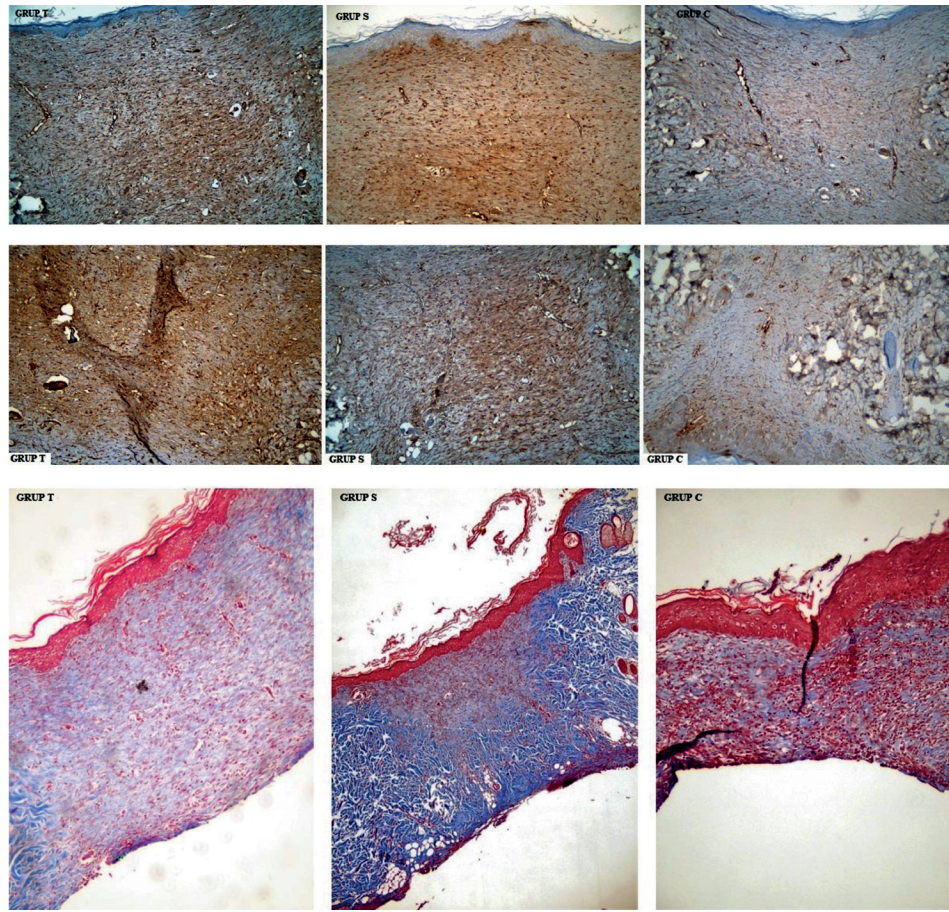


Figure 4. First row represents the microscopic image with immunohistochemical CD31 antibody applied to evaluate microvascular structure (x100). Microvascular structures appear to be reduced in Group T compared to Group S and Group C. Second row represents the microscopic image with immunohistochemical CD34 antibody applied to evaluate angiogenesis (x100). Similar to CD31 antibody, vascularity was decreased in Group T compared to other groups. Third row represents the microscopic image by Masson's Trichrome staining method to evaluate fibrosis intensity (x40). Fibrosis density appears to be higher in Group S than in the other two group

Table 3. Evaluation of difference in immunohistochemical marker characteristics by groups

	Group T (n=9) $\bar{x} \pm SD$	Group S (n=9) $\bar{x} \pm SD$	Group C (n=9) $\bar{x} \pm SD$	p-values	p-values Group T-Group S Group T- Group C Group S- Group C
CD34/mm ²	7.89±4.17	11.44±2.65	12.89±5.13	0.04*	0.18** 0.04** 0.74**
CD31/mm ²	6.56±3.71	12.33±3.39	15.44±4.58	<0.001*	0.01** <0.001** 0.23**
VEGF/mm ²	8.22±2.99	13.89±3.85	12±5.12	0.02*	0.02** 0.14** 0.6**
CD68/HPF	13.12±5.89	10.11±3.29	10.78±2.11	0.29*	

Data are shown as mean±standard deviation. The different superscripts over p-values (*,**) indicates the utilized statistic test respectively One Way ANOVA test, Post Hoc Tukey. Statistically significant p-values are marked in bold font.

Table 4. Evaluation of the difference status of the characteristics by groups.

	Group T (n=9) $\bar{x}\pm SD$	Group S (n=9) $\bar{x}\pm SD$	Group C (n=9) $\bar{x}\pm SD$	p-values	p-values Group T-Group S Group T-Group C Group S-Group C
Epidermis thickness (μm)	92.19 $\pm$ 31.09	50.18 $\pm$ 5.41	91.02 $\pm$ 7.83	<0.001*	<0.001** 0.99** <0.001**
Granulation tissue thickness (mm)	1.2 $\pm$ 0.45	1.59 $\pm$ 0.48	1.13 $\pm$ 0.37	0.08*	-
Tissue thickness (mm)	1.58 $\pm$ 0.43	2.11 $\pm$ 0.62	1.7 $\pm$ 0.32	0.06*	-
Granulation tissue thickness/tissue thickness	0.78 $\pm$ 0.24	0.76 $\pm$ 0.12	0.66 $\pm$ 0.11	0.28*	-

Data are shown as mean $\pm$ standard deviation. The different superscripts over p-values (*,**) indicates the utilized statistic test respectively One Way ANOVA test, Post Hoc Tukey. Statistically significant p-values are marked in bold font

DISCUSSION

Chronic ischemic wounds present complex challenges in terms of monitoring and treatment, often encountered by various medical specialties, including internal medicine, surgery, and particularly in settings such as intensive care units and palliative care units. Tramadol hydrochloride is used in many different commercial forms, especially in the form of extended-release tablets and pain pumps, in the treatment of pain caused by acute and chronic wounds in today's clinical practice. In our study, we examined the effect of systemically administered tramadol hydrochloride on wound healing. We showed that intraperitoneal administration of tramadol hydrochloride to rats with an ischemic wound model positively affected the acute phase of wound healing in rats.

Various studies have proven that the number of endogenous opioids increases in the wound area and plasma during injury. These studies demonstrated that the opioid receptor system has the potential to alter immune function in response to injury [16]. There are basically 4 main receptors in the opioid receptor family: delta, kappa, nociceptin, and mu (MOPr). Endogenous ligands that bind to MOPr have been shown to upregulate the production of cytokeratin-16, which is not found in healthy skin but can only be seen in conditions such as skin cancer and hyperproliferative skin diseases [17]. This has been accepted as evidence that MOPr plays a role in wound healing [16-18]. The active metabolite M1 of tramadol hydrochloride has proven to be the metabolite with the highest affinity for MOPr [19]. As a result of our study, a significant difference was found in wound healing in the group in which tramadol hydrochloride was applied systemically compared to the control and sham groups. We think that this positive effect on wound healing may be due to the agonist effect of M1.

In a study, Ozkan et al. investigated the effects of tramadol hydrochloride infiltration into the surgical wound incision line

on wound healing in rats and proved that a 2.5% concentration of tramadol hydrochloride infiltration application did not have a negative effect on wound healing [12]. In a similar study by Hanci et al., tramadol hydrochloride infiltration was applied to the surgical wound area in order to benefit from its local anesthetic effect, and its effects on wound healing were compared with those of local anesthetic agents [11]. As a result of this study, it was stated that tramadol hydrochloride did not adversely affect the wound healing process, and there was no significant difference from the control group in terms of other parameters evaluating wound healing. However, tramadol hydrochloride, a prodrug, can be thought to exert its effects on wound healing through its active metabolite. In our study, tramadol hydrochloride was administered systemically, and the effects of its active metabolite were investigated. Further studies are needed with its active metabolite, M1 to investigate its effect in local applications.

In a recent study, Feng et al carried out studies on angiogenesis on human umbilical vein endothelial cells to evaluate the effects of different opioid agents on the endothelium and showed strong proangiogenic activity of morphine [20]. The effects of morphine, which has a strong proangiogenic effect, on wound healing have been demonstrated in in vivo experiments, but in the study of Rook et al. on rats, there was no difference in the duration of wound healing in rats treated topically with morphine compared with the control group [10]. Literature studies on the angiogenic effects of tramadol hydrochloride show conflicting results. Karaman et al, in their angiogenesis study on the chorioallantoic membrane model, observed that tramadol hydrochloride had anti-angiogenic effects at a dose of 10 μM [21]. In contrast, Ozgurbuz et al. demonstrated that tramadol hydrochloride significantly increased VEGF expression in specific colon cancer cell lines when compared to the control group. They suggested that tramadol hydrochloride might potentially influence angiogenic potential [22]. In our study, three different immunohistochemical

vascular markers were used to evaluate angiogenesis, and the results were interpreted against tramadol hydrochloride. In contrast to the study of Ozgurbuz et al, samples belonging to the tramadol group showed lower expression of VEGF, suggesting that tramadol hydrochloride may suppress the angiogenic potential. The results of CD34 and CD31 antibodies, which are other vascular markers studied, are also compatible with VEGF results. When the results of the sham group and the control group are examined, there is no statistically significant difference, which shows us that the micro vascularity suppressive effect of tramadol hydrochloride is independent of its solvent. Further studies are needed to evaluate the hypothesis that pure tramadol hydrochloride will suppress angiogenesis.

As a result of our study, considering the differences in epidermis thickness although the thickness of the epidermis was low in the Group S, the significant difference in the thickness of the epidermis in the Group T suggests that tramadol hydrochloride may have an increasing effect on epithelialization. However, it is not possible to make a clear interpretation as to whether this effect will predispose to keloid formation. This hypothesis can be examined more clearly with a study using pure tramadol hydrochloride.

CONCLUSION

The aim of our study is to evaluate the effects of systemically administered tramadol hydrochloride on the healing of ischemic wounds, and in our literature review, we could not find any studies evaluating the histopathological and macroscopic effects of tramadol hydrochloride on ischemic wounds and wound healing.

As a result of our study, we determined that tramadol hydrochloride accelerated the physical healing of ischemic wounds in rats in the acute period. Tramadol Hydrochloride is an agent that is frequently used for purposes such as analgesia and even local anesthesia in current anesthesia and intensive care practice. After the inspiring results of our study, we believe that in the coming years, it will provide comfort to ischemic scar tissue by accelerating wound healing and controlling the pain caused by ischemic wounds.

Limitations

This study employed animal models, which, although necessary and valuable for initial investigations, do not always perfectly replicate human physiological responses. Consequently, the findings may not be directly translatable to human subjects, and further research involving human trials is necessary to validate these results. Furthermore, the study focused on specific immunohistochemical markers and wound healing parameters, which, while comprehensive, do not encompass all aspects of wound healing and immune response. Other factors and markers could provide a more holistic understanding of the healing process.

Ethics Committee Approval: Ethics Committee with the number ETIK 2022/02 on January 27, 2022, from the Health Sciences University Gülhane Animal Experiments Local Ethics Committee, the animals were obtained from the Health Sciences University R&D Center Gulhane Experimental Animals Production and Research Unit.

Patient Consent for Publication: Since our study is an animal experiment, there is no patient consent form.

Data Sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions: All authors contributed equally to the article.

Conflict of Interest: The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

Funding: This study received funding from Health Sciences University Scientific Research Projects Coordinatorship with project number 2022/139.

REFERENCES

1. Smyj R, Wang XP, Han F. Tramadol hydrochloride. Profiles Drug Subst Excip Relat Methodol. 2013;38:463-94.
2. Bravo L, Mico JA, Berrocoso E. Discovery and development of tramadol for the treatment of pain. Expert Opin Drug Discov. 2017;12:1281-91.
3. Miotto K, Cho AK, Khalil MA, Blanco K, Sasaki JD, Rawson R. Trends in tramadol: pharmacology, metabolism, and misuse. Anesth Analg. 2017;124:44-51.
4. Lai J, Ma SW, Porreca F, Raffa RB. Tramadol, M1 metabolite and enantiomer affinities for cloned human opioid receptors expressed in transfected HN9.10 neuroblastoma cells. Eur J Pharmacol. 1996;316:369-72.
5. Vazzana M, Andreani T, Figueiro J, Faggio C, Silva C, Santini A, et al. Tramadol hydrochloride: pharmacokinetics, pharmacodynamics, adverse side effects, co-administration of drugs and new drug delivery systems. Biomed Pharmacother. 2015;70:234-8.
6. Guest JF, Ayoub N, McIlwraith T, Uchegbu I, Gerrish A, Weidlich D, et al. Health economic burden that different wound types impose on the UK's National Health Service. Int Wound J. 2017;14:322-30.
7. Kimura S, Tsuji T. Mechanical and immunological regulation in wound healing and skin reconstruction. Int J Mol Sci. 2021;22:5474.
8. Cuéllar B, Fernández AP, Lizasoain I, Moro MA, Lorenzo P, Bentura ML, et al. Up-regulation of neuronal NO synthase immunoreactivity in opiate dependence and withdrawal. Psychopharmacology (Berl). 2000;148:66-73.
9. Poonawala T, Levay-Young BK, Hebbel RP, Gupta K. Opioids heal ischemic wounds in the rat. Wound Repair Regen. 2005;13:165-74.
10. Rook JM, Hasan W, McCarron KE. Temporal effects of topical morphine application on cutaneous wound healing. Anesthesiology. 2008;109:130-6.

11. Hancı V, Hakimoğlu S, Özaçmak H, Bektaş S, Özaçmak HS, Özdamar SO, et al. Comparison of the effects of bupivacaine, lidocaine, and tramadol infiltration on wound healing in rats. *Rev Bras Anesthesiol.* 2012;62:799-810.
12. Ozkan D, Seker D, Ergil J, Yalcindag A, Han U, Ginis Z, et al. The effects of tramadol infiltration on wound healing in rats. *Acta Chir Belg.* 2013;113:434-8.
13. 2021 Indiana University Bloomington Laboratory Animal Resources Guidelines: Guidelines for Anesthesia and Analgesia in Rats. Available at: <https://lar.indiana.edu/policies-guidelines/Guidelines-for-Anesthesia-and-Analgesia-in-Rats-FINAL.docx> Accessed November 14, 2021.
14. Trujillo AN, Kesl SL, Sherwood J, Wu M, Gould LJ. Demonstration of the rat ischemic skin wound model. *J Vis Exp.* 2015:e52637.
15. Norman G. Likert scales, levels of measurement and the "laws" of statistics. *Adv Health Sci Educ Theory Pract.* 2010;15:625-32.
16. Bigliardi-Qi M, Bigliardi P. The roles of opioid receptors in cutaneous wound healing. *Handb Exp Pharmacol.* 2018;247:335-45.
17. Bigliardi-Qi M, Bigliardi PL, Eberle AN, Büchner S, Ruffli T. Beta-endorphin stimulates cytokeratin 16 expression and downregulates mu-opiate receptor expression in human epidermis. *J Invest Dermatol.* 2000;114:527-32.
18. Gerritsen MJ, Elbers ME, de Jong EM, van de Kerkhof PC. Recruitment of cycling epidermal cells and expression of filaggrin, involucrin and tenascin in the margin of the active psoriatic plaque, in the uninvolved skin of psoriatic patients and in the normal healthy skin. *J Dermatol Sci.* 1997;14:179-88.
19. Gillen C, Haurand M, Kobelt DJ, Wnendt S. Affinity, potency and efficacy of tramadol and its metabolites at the cloned human mu-opioid receptor. *Naunyn Schmiedebergs Arch Pharmacol.* 2000;362:116-21.
20. Feng T, Zeng S, Ding J, Chen G, Wang B, Wang D, et al. Comparative analysis of the effects of opioids in angiogenesis. *BMC Anesthesiol.* 2021;21:257.
21. Karaman H, Tufek A, Karaman E, Tokgoz O. Opioids inhibit angiogenesis in a chorioallantoic membrane model. *Pain Physician.* 2017;20:SE11-21.
22. Özgürbüz U, Gencür S, Kurt FÖ, Özkalkanlı M, Vatansever HS. The effects of tramadol on cancer stem cells and metabolic changes in colon carcinoma cells lines. *Gene.* 2019;718:144030.