# DEVELOPMENT OF A TRANSDERMAL SONOPHORESIS DEVICE FOR DELIVERY OF TRAMADOL IN RATS

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DEVELOPMENT OF A TRANSDERMAL SONOPHORESIS DEVICE FOR DELIVERY OF TRAMADOL IN RATS

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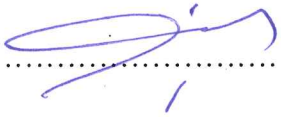
Assist. Prof. Dr. Çiğdem Altınok ..... 

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# ABSTRACT


## DEVELOPMENT OF TRANSDERMAL SONOPHORESIS DEVICE FOR DELIVERY TRAMADOL IN RATS

The main purpose of this study was to design a sonophoretic device to penetrate the model drug tramadol from rat skin. For this study, we demonstrated that tramadol sonophoresis is safe and significantly increased analgesia when compared with the control group, which were given tramadol without sonophoresis application. Sonophoresis application to rats showed a statistically significant difference between the control group and intraperitoneal (ip) application of the drug, $p < 0.05$. Rats with 28 mg/kg tramadol hydrogel were applied with sonophoresis at a frequency of 40 KHz for up to 60 minutes.

16 rats were randomly divided into four groups. These were control group, ip tramadol injected group, tramadol hydrogel group and tramadol hydrogel with sonophoresis application group. We have concluded that the device worked correctly and safely with the sonication application. Low-frequency sonophoresis did not cause either burns or erythematous streaks.

# ÖZET


## TRANSDERMAL SONOFOREZ CİHAZININ SIÇANLARDA TRAMADOL HİDROJEL SALINIMININ GELİŞTİRİLMESİ

Bu çalışmanın temel amacı, sonoforetik cihazın, etken madde seçilen "tramadol hidrojelin" sıçan derisinden geçirilerek deri altına iletimi için tasarlanmasıdır. Bu çalışma ile yöntemin güvenilir olduğunu kanıtlamış olduk. Sonoforez uygulaması kontrol grubu ve intraperitoneal (ip) uygulama ile karşılaştırıldığında istatistiksel olarak anlamlı bir farklılık olduğunu gördük, $p < 0.05$. Sonoforetik uygulamalar, sıçan derisine 28 mg / kg tramadol hidrojel ile 10, 20, 30, 40 ve 60 dakika süre ile uygulandı. Analjezik eşik ölçümü 'sıcak plak testi' ile 5 farklı zaman aralığında gerçekleştirildi.

16 tane sıçan dört farklı gruba ayrıldı. Bu gruplar; kontrol grubu, ip tramadol grubu, tramadol hidrojel grubu ve tramadol hidrojelin sonoforez eşliğinde uygulandığı gruptur. Sonuçların istatistiksel analizi Kruskal Wallis ANOVA testi kullanılarak yapıldı. Test sonuçları, ortalama ± standart sapma olarak ifade edildi. 0.05'ten küçük olan P değerleri anlamlı sonuç olarak kabul edildi. Sonoforez uygulamasını doğru ve güvenli bir şekilde sonuçlandırdık. Bu uygulama ile, sıçanlarımızda herhangi bir rahatsızlık durumunun veya cilt yanıklarının olmadığı gözlendi.

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| | |
|---|---|
| Ω | Ohm |
| °C | Centigrade |
| J/ $Cm^2$ | Joule/Square centimeter |
| U/ml | Unit/Milliliter |
| | |
| CNS | Central nervous system |
| MIN | Minute |
| KHz | Kilohertz |
| MHz | Megahertz |
| MW/ $Cm^2$ | Microwatt/Square centimeter |
| Ms | Millisecond |
| SC | Stratum corneum |
| Tr Hc | Tramadol hydrochloride |

# 1. INTRODUCTION

## 1.1. TRANSDERMAL DELIVERY SYSTEMS: SONOPHORESIS

Transdermal delivery of drugs has many advantages. Transdermal drug delivery systems which are oral and injection methods compared to conventional delivery systems, it is seen many advantages. Transdermal delivery has become popular application for medical studies. Because transdermal drug delivery has many advantages. These advantages are: it increases bioavailability, can be used for long-term treatments of chronic illness, continued maintenance of plasma drug levels, decreased adverse drug effects, enhanced patient compliance due to reduction in number and frequency of doses, less damage to tissues and effective costly. Transdermal drug delivery techniques use a patch. Patch contains drug substance and it is non-invasive method (without needle), convenient, and painless and can avoid gastrointestinal toxicity which is called peptic ulcer disease, degradation by gastrointestinal tract (e.g. polypeptides such as insulin) and the hepatic first pass metabolism. It allows to participate in the systemic circulation through the skin controlled delivery. In Figure 1.1 seen that skin layers can be penetrated with transdermal drug delivery. Stratum corneum is the barrier for drug delivery. Sonophoresis technique is the physical parts of transdermal drug delivery. Sonophoresis provides to penetrate from stratum corneum absorption.

First published article on sonophoresis is in early 1950s by Fellinger and Schmidt for the treatment of polyarthritis. They applied hydrocortisone with ultrasound ‘’massage’’ technique on the hand’s digital joints. This technique provided us with better results considered to hydrocortisone injections for bursitis treatment[1]. Sonophoresis can also be applied on a variety of drugs which have capability to assist the penetration as well. One of the most important applications of this method is for local anesthetics transdermal application[2]. Sonophoresis is a special technique which increases transdermal penetration of molecules by using ultrasound waves to deliver drugs through the skin. Sonophoresis can also be applied on a variety of drugs which have capability to assist the penetration as well.

The aim of this study was to set sonophoresis drug delivery model and examine it in laboratory on rats.

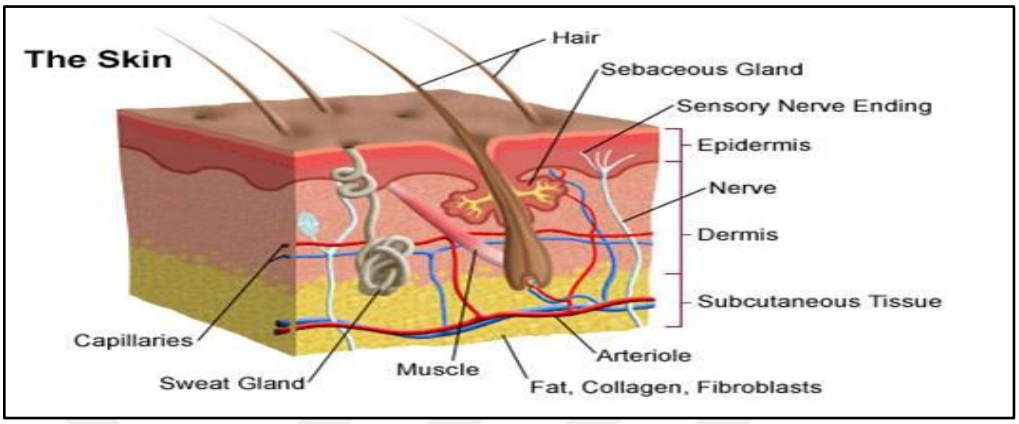


Figure 1.1. Skin layers [1]

## 1.2. LOW-FREQUENCY SONOPHORESIS

Biotechnology has its milestone on low-frequency sonophoresis which has studied by scientist for the last 10 years. Sonophoresis provides electrical energy to turn into mechanical energy or vice versa. As seen as in Figure 1.2 low-frequency sonophoresis helps to increase diffusion and ultrasound waves causes convection. The most powerful feature of low-frequency sonophoresis is measuring frequency and drug delivery ratio which can be controlled by an ultrasonic transducer. It can help to hospital staff members for delivery drug with controllable way.

Besides, low-frequency sonophoresis helps to delivery of low and high molecular (macromolecule like heparin and glucose) of drugs which includes hydrophilic drugs. Hence, it has an important technique for drug delivery systems.

Low- frequency sonophoresis presents advantages over other transdermal delivery methods. It can be tested by application time and ultrasound parameters[3, 4]. So it provides local delivery[5]. The other advantage is that it can be controlled by varying frequency and intensity of ultrasound[6]. In other advantage is that it can also be used with

drug-containing patch. It is the effective release of prescribed medications cannot be easily achieved by conventional patches, since the dose may be discharged or released. For the solution of this problem, controlled therapeutic systems are preferred by physical means. Therefore, it provides to penetrate drug whenever it necessary from patients. It can monitor blood analyses as well as blood glucose for diabetes[7].

Low frequency sonophoresis was used for therapeutic purposed with help of piezoelectric disc. It is formed by the addition of mechanism. As a result of rapid change in voltage with transducer motion. It consists of high frequency pressure wave(ultrasound). The active substance is from the ultrasound device. It is provided by a contacting agent which transmits energy to the skin. Ultrasound waves with mechanical changes in the skin resulting from the stratum corneum. Cavitation (cavity formation) occurs in keratinocytes and resulting cavitation cell increased permeation, rapidly reversible cell damage.

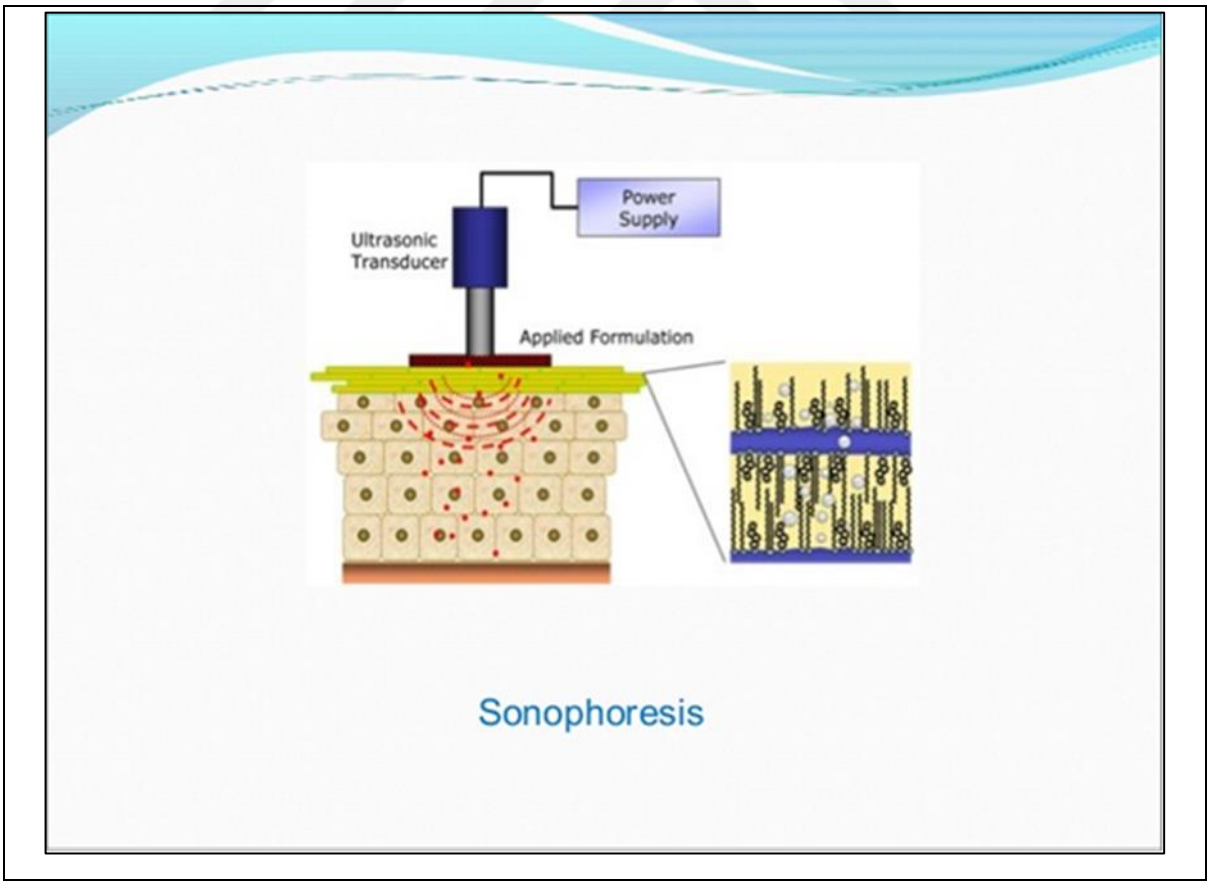


Figure 1. 2. Sonophoresis mechanism [7]

## 1.3. DEPENDENCE OF TRANSPORT ON ULTRASOUND PARAMETERS

Ultrasound links to skin barriers. These are stratum corneum thickness, high skin impedance, low skin hydration, low useable area for solid transportation, age, blood flow, follicles such as sweat and hair, trauma on skin, humidity and temperature, presence of chemicals and chronical usages of drugs.

There are four main ultrasound parameters which are frequency, intensity, duty cycle, and application of time. Low-frequency sonophoresis has an extensive study on the dependence of permeability enhancement on frequency and intensity in the low-frequency which has been shown by Tezel et al[8].

### 1.3.1. The Frequency

Emitted wave frequency is related to the size of the crystal. Attenuation of an acoustic wave is inversely proportional to its frequency. If the frequency increases, ultrasound penetrates into less deeply under the skin. High frequencies range from 1-3 MHz. High ferquencies were first surveyed as physical enhancers for transdermal delivery of drugs[9]. Low frequency ranges from 20 to 100 kHz.

## 1.4. MECHANISMS OF LOW-FREQUENCY SONOPHORESIS

Many of variables affect low-frequency sonophoresis. These are cavitation (pore induction), frequency, amplitude, intensity and application of time.

### 1.4.1. Cavitation

Low-frequency sonophoresis has an important breakthrough on the formation and collapse of gaseous cavities(acoustic)[10] . Cavitation means the collapse and formation of gas bubbles in a liquid environment and the resulting collapse when exposed to a sound wave

in such an environment. Cavitation is overwhelmingly meet with the coupling medium (the liquid consists in between the ultrasound transducer and the skin. The frequency and acoustic pressure amplitude are related to the maximum radius of the cavitation bubbles. During low-frequency sonophoresis, cavitation occurs within 15 micrometers of SC, and in order to overcome this, inert cavitation is created in the skin layers. Moreover, with the usage of acoustic spectroscopy, quantifying inertial cavitation has become more handy[11]. It can produce violent micro streams, which goes up the bioavailability of the drugs[12] .Cavitation happens because of the nucleation of small gaseous cavities during the negative pressure cycles of ultrasound. As a result, cavitation provides the disordering of the lipid bilayers and formation of aqueous channels provides to penetrate easily in the skin [13].

### 1.4.2. Convection

Convection is a significant factor for low-frequency sonophoresis. Acoustic streaming (convective process) can be augment the bioavailability of drugs such as Lidocaine.

Levy et al. demonstrated that when convection and cavitation were mixed with mannitol, inulin, they enhanced delivery to skin[14].

### 1.4.3. Thermal Effects

Attenuation of ultrasound wave leads to thermal increasement for low-frequency sonophoresis. Ultrasound waves cause heating of the medium. Thermal effects cause to increase skin permeability. It provides to increase kinetic energy and diffusion of drugs, dilates points of entry of the skin, promotes drug absorption and enhances circulation of blood for in-vivo experiments. Duty cycle and ultrasound intensity are parameters which are directly related to thermal effects. Therefore, it is important for low-frequency sonophoresis application.

## 1.5. SYNERGESTIC EFFECT WITH OTHER ENHANCERS

Ultrasound application is not effective compared to usage of low-frequency ultrasound combinations with other enhancers have been shown to be more efficient. Moreover, increasing transdermal transport, especially with the combination of ultrasound with other enhancers causes to diminish the enhancers needed to help the drug flux. Therefore, combination of ultrasound with other enhancers will definitely increase the reliability with decreasing the strength of selected enhancers.

### 1.5.1. Ultrasound and Chemicals

Mitragotri et al. carried out a work of the synergistic effect of low-frequency ultrasound that is using 20 kilohertz with sodium lauryl sulfate. It has been shown that the administration of sodium lauryl sulfate causes an approximately 3-fold increase in mannitol permeability and is only about 8 times greater than that of ultrasound for 90 minutes. It was also observed that the induced sulphate solution increased approximately 200-fold in the skin permeability of mannitol.

In particular, with the insufficiency of surfactants, the threshold ultrasound energy was about 141 Joules / $cm^2$ to produce a detectable change in skin impedance. The addition of 1% sodium lauryl sulfate to the solution reduced the threshold to about 18 Joules / $cm^2$. The various results of this synergistic effect indicated that low frequency ultrasound indicated better spread and diffusion of the surfactant in the skin.

### 1.5.2. Ultrasound and Iontophoresis

The synergy between low frequency ultrasound and iontophoresis is of great importance as it increases transdermal transport. In fact, this combination is particularly useful in the treatment of transdermal transport by Lee et al. By using heparin as a model drug, it has been shown to have a better and easier way to investigate the synergistic effect of

ultrasound and iontophoresis on transdermal transport. Approximately 10 minutes prior to the administration of iontophoresis, the skin was once treated with 1% dodecyl pyridinium chloride solution. As a result, the increase in heparin flux of ultrasound and iontophoresis applications was recorded approximately 56-fold increased with these applications.

## 1.6. EQUIPMENT AND DEVICES

### 1.6.1. Tramadol Pharmacology

Tramadol is a centrally acting analgesic agent with μ-opioid agonist properties, blocks nor adrenaline uptake. Tramadol hydrogel is a similar molecule with 4-phenyl-piperidine analogue of codeine, which is also acting as analgesic and painkiller. It can be used by patients in the orthopedics spine clinic and may even be beneficial in patients with poor cardiopulmonary function, including patients with older people, obese and smokers, patients with liver or renal dysfunction and nonsteroidal anti-inflammatory patients. It can be also used post-operative pain relief. It was chosen because it has advantages. Its elimination half life is about 6 hours.

Tramadol has a high solubility in the oral cavity. It is also known as Tramadol Hydrochloride (Tr HC) and has opioid and non-opioid properties. It is primary effective on the central nervous system (CNS). This drug is similar to codeine and morphine as considered to structurally. However, it is 6000 times less active than morphine and is 10 times less effective than codeine. However, in 1995, it was rated as a treatment of acute pain with food and drug administration (FDA). Tramadol hydrochloride effects on low-affinity m-opioid and k-opioid receptors, and norepinephrine (NE), blocking monoamine receptor systems. It provides serotonin (5-HT) reuptake due to inhibition of pain distribution in the spinal cord[15]. It has also  a lower incidence of adverse effects. It is used for many patients for analgesia effect.

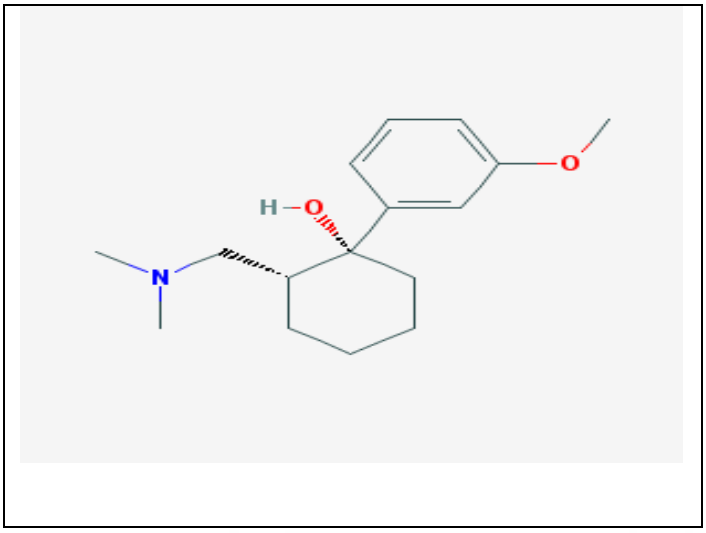


Figure 1. 3. Tramadol structure

### 1.6.2. Hot Plate Analgesia Test

The hot plate is one of the most widely used tests. Because it is helpful to determine the analgesic efficacy of experimental drugs in rodents. In our experiment we used the guidelines developed by Ankier S.I. (1974)[16]. A hot plate has been adjusted to 54°C and the latency of the first reaction (licking of the paws or jumping response- a jump has been identified by all 4 paws leaving the heated surface-) has been recorded. A cut off period of 60 seconds has been considered to avoid any damage to the paws. Rats were placed on the hot plate one by one and response latency was measured with a stopwatch. Observations showed that the majority of animals reacted to the heat by licking their paws[17].

# 2. MATERIALS AND METHODS

## 2.1. DEVELOPMENT OF TRANSDERMAL SONOPHORESIS DEVICE

Transdermal sonophoresis device was developed at Yeditepe University biomedical engineering laboratories.

Lm555 oscillator has been used for this experiment. On this experiment, aim is to produce square wave pulses provided continuously by the 555 IC. On the other hand, the 555 timer IC has connected either in its monostable mode therefore it generates a back and front type switching action. Connection of the 555 timer IC in an unstable mode is a tricky part. When it was sought highly precise free roaming waveform, very stable 555 oscillator has to used. Also, RC circuit has to be connected to oscillator which contains 2 resistors and capacitors. The 555 oscillator can be used which generates stabilized square wave output waveforms. Its duty cycle is between 50-100%.

The device has stopped working until for the next trigger pulse. It initiates to act as an unstable multi vibrator. It has a great importance to continuously re-trigger effect of circuit. Pin 2 which is trigger input provides triggering process connecting to 555 timer and threshold input to pin 6 acts as an unstable oscillator with together. Single timing resistor has a key act on this device because it has been split into two different resistors which are R1 and R2. Pin 7 which is discharge input has been linked to their junctions.

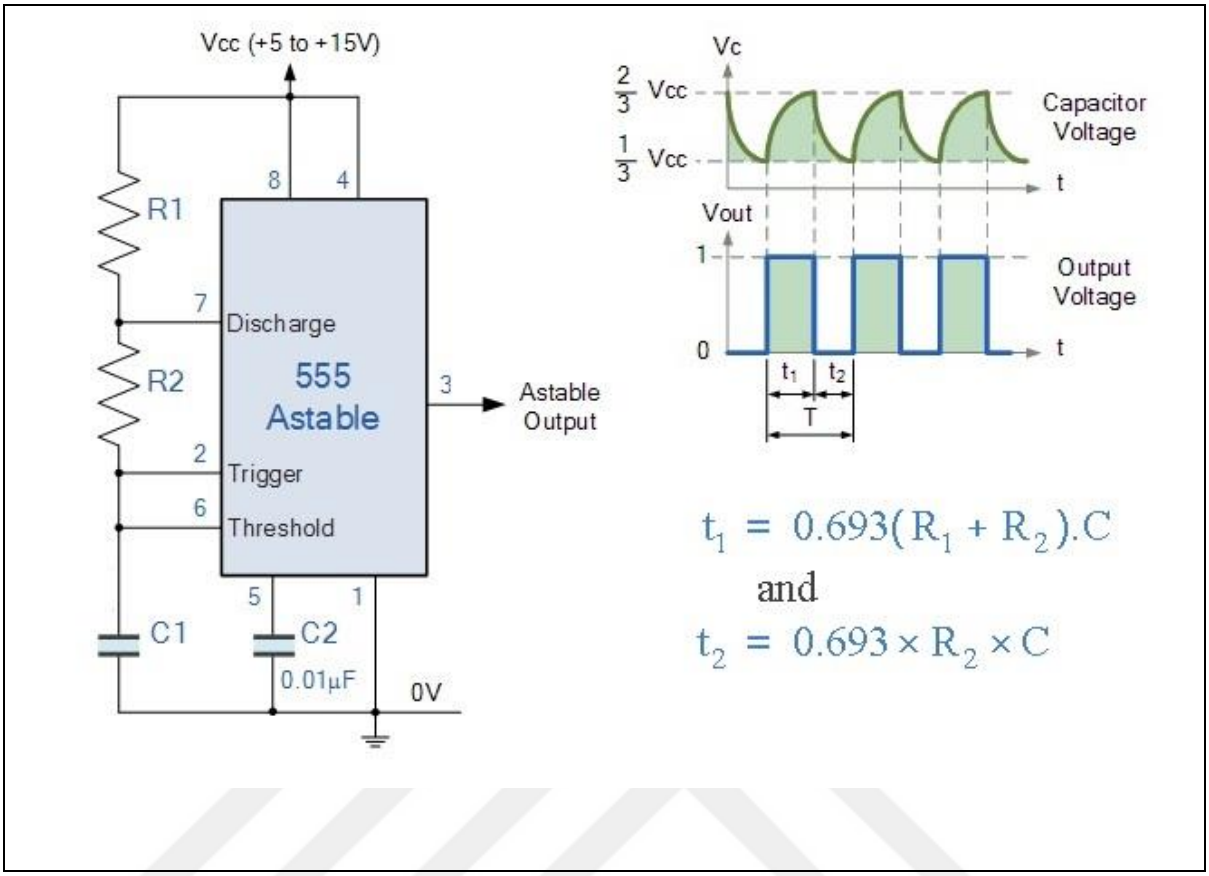


Figure 2. 1. Sonophoresis device circuit [18]

Firstly, charge and discharge times which means period were calculated for 40 Kilohertz ultrasonic transducer. Period was calculated to 1.1 equation.

$$t_1 = (1/40.000 \text{ Hertz}) = 25 \text{ microsecond} = t_2 \quad (1.1)$$

$$t_{1=}\, t_{2=}\, 12.5 \text{ microsecond} \quad (1.2)$$

Then the capacitor charges up to 2/3Vcc which is the upper comparator limit determined by below formula:

$$t_{1=}\, 0.693*(R1+R2)\,*C \quad (1.3)$$

It is combination and discharges itself down to 1/3Vcc means the lower comparator limit determined by below formula:

$$t_{2=}\, 0.693*(R2*C) \quad (1.4)$$

Where, R is in Ω and C in Farads.

Resistance1 and resistance2 were found 1000 kiloohm. Capacitance was found 1 microfarad.

This outcomes in an output waveform whose voltage level is approximately equal to $V_{cc}$ is calibrated to 15V. ON and OFF time periods were determined by the capacitor and resistors combinations. The discharge cycle of the output was calculated and it required a full charge of individual durations. It was obtained from this formula:

$$T= t_{1+}t_2= 0.693*(R1+2R2)*C \quad (1.5)$$

The output frequency of oscillations can be found by reversing the equation above for the total cycle time. It gives a final equation for the output frequency of an unstable 555 Oscillator below formula:

$$f=1/T=1.44/(R1+2R2)*C \quad (1.6)$$

Duty Cycle as known the "Mark-to-Space" ratio. It is the output waveform can be certainly fixed. It is the ratio of resistor R2 to resistor R1. The Duty Cycle for the 555 Oscillator, which is the ratio of the ON time divided by the OFF time was calculated Duty Cycle formula:

$$\text{Duty Cycle}= Ton/ (Ton+Toff)= (R1+2R2)* C \quad (1.7.)$$

The duty cycle has no units because it is a ratio. However, it can be expressed as a percentage. Timing resistors are equal so the output duty cycle will be 2 divided by 1. So result is 66% ON time and 33% OFF time as far as the period.

### 2.1.1. The Background of Proposed Sonophoresis Device

The backside of my device is ultrasonic transducer principle. It provides to convert electrical energy to acoustic energy and vice versa. The conversion of electrical energy to acoustic (and vice versa) is a function of the piezoelectric element. IRF640 n-channel metal oxide semiconductor field effect (MOSFET) was used. In n-channel enhancement MOSFET a lightly doped p-type substrate forms the body of the device and source and drain regions are heavily doped with n-type impurities. Two ultrasonic transducers which is 40 Kilohertz were used as a receiver and transmitter principle. Ultrasonic transducers were tested until obtained at 40 Khz on oscilloscope. Firstly, 73 kHz was seen on screen but later it was obtained 40 kHz using ultrasonic distance sensor test. It was not observed on the first test because resistances had different value on tolerance. It is usually used a magneto strictive and piezoelectric ceramic type. Ultrasonic transducers have many advantages. These are high level amplitude, low heat transmission, high efficiency application, low cost, fast delivery and stable output anaffected by variations in load. Arduino Uno was used for checking our device working. It is a development board based on a microcontroller and its programmable with C language. At the last step, when finished tests, all of the circuit elements were solded on perforated plaque.

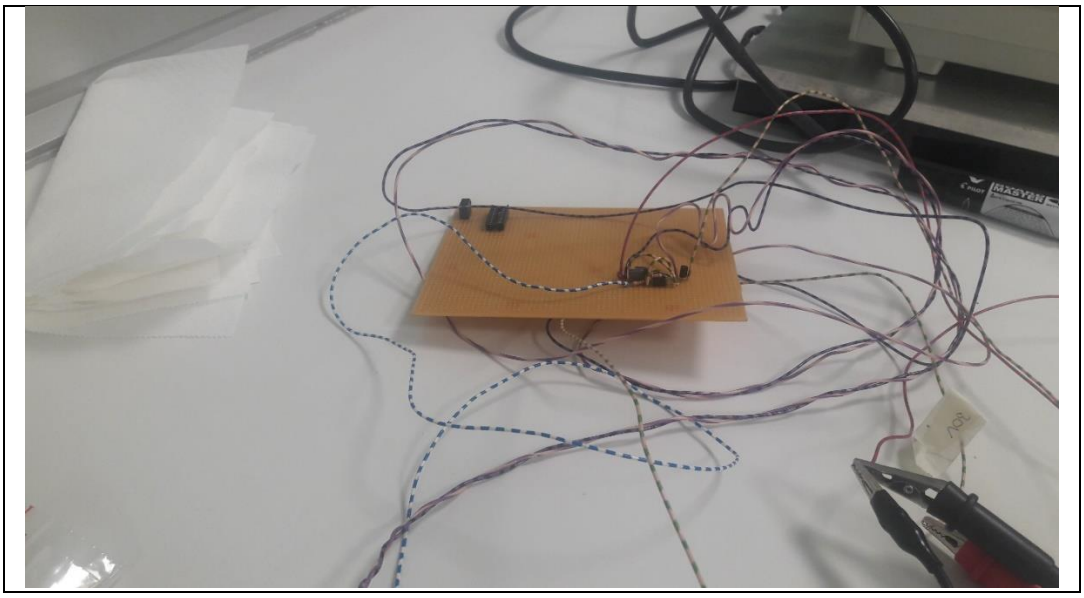

Figure 2.2. Designed sonophoresis circuit

## 2.2. GEL PREPERATION

### 2.2.1. Hydrogel Formulation

Hydrogels are three-dimensional, hydrophilic, porous and soft consistency. Hydrogels are generally prepared based on hydrophilic monomers. In this study, firstly 20 grams of Pluronics F 127 was weighed and dispersed into the 40 mL purified water. The dispersed polymer was put into the refrigerator overnight and was dissolved homogenously as a hydrogel. Tramadol solution was incorporated into hydrogel.

### 2.2.2. Hot Plate Analgesia Test

We determined four groups for measure the analgesia effect on rats. The first group were chosen as control group. 3 rats were placed without sonication and tramadol hydrogel at 0, 10, 20, 30, 40 and 60 minutes. We noted on each time jumping or paw licking response as seen as in Figure 2.2. The second group is tramadol hydrogel (GT) group. In this group, only tramadol hydrogel was put on rats and then their paw licking was noted according to hot plate analgesia test. Before the experiment, the rats were turned on their backs, and their chest and lower body parts were shaved. Then, tramadol hydrogel was put on rats.

The third group is intraperitoneal (ip) group. The last group is sonication application (GTS). Our designed sonication device was applied on rats. In this procedure, tramadol hydrogel was put on rats and 40 kHz ultrasonic transducer were put on rats. In this experiment set up, power supply was used for supply voltage. Voltage was fixed to 15Volt. 1 minute was determined for cut-off response. After 10 minutes rat was put on hot plate and noted jumping or paw licking. This procedure, repeated on each time intervals 10, 20, 30, 40 and 60 minutes.

In the ip group, each of rats were weighed and they are approximately 250-300 grams. We calculated tramadol dosage key to the total weight of rats were calculated by 10%. Hence, at this calculation was resulted 28 mg/kg per body weight. Then, tramadol was injected to

3 rats. Every process performed in the experiments was conscientiously calculated and successfully completed under the most favorable conditions. To sum up, model drug of tramadol analgesic latency on response increased under each condition and especially with sonication application.

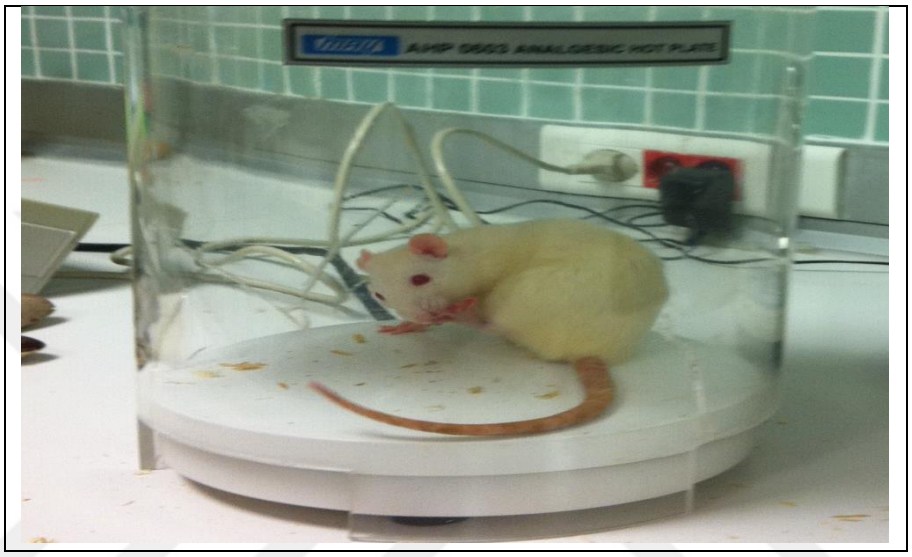

Figure 2. 3. Hot plate analgesia sensitivity test (Spraque Dawley rat licking of the paws)

### 2.2.3. Animal Study

The ethical permissions were taken from Yüdetam at Yeditepe University. 16 rats which type is Spraque Dawley male rats were used in this project as seen as below in Figure 2.3. Their ages were approximately 3 months. They were not killed after the experiment. Any anesthesia material was not applied on rats during the experiment.

Figure 2. 4. Spraque Dawley 6 months male rats

# 3. RESULTS

## 3.1. RESULTS OF DEVELOPED SONOPHORESIS DEVICE

Sonophoresis device results were obtained from hot plate analgesia sensitivity test from rats. 40 kHz provided enough penetration for analgesia test. It was applied on rats and obtained time for latency response. Designed sonophoresis device provided to increase absorption analgesia effectively.

## 3.2. HOT PLATE LATENCY TEST RESULTS

Hot plate latency test was compared to 4 groups. These are control, tramadol hyrogel and tramadol hydrogel with sonication group and intraperitoneal(ip) group. The obtained hot plate latency test results, the graph was plotted as seen as in Figure 3.1.

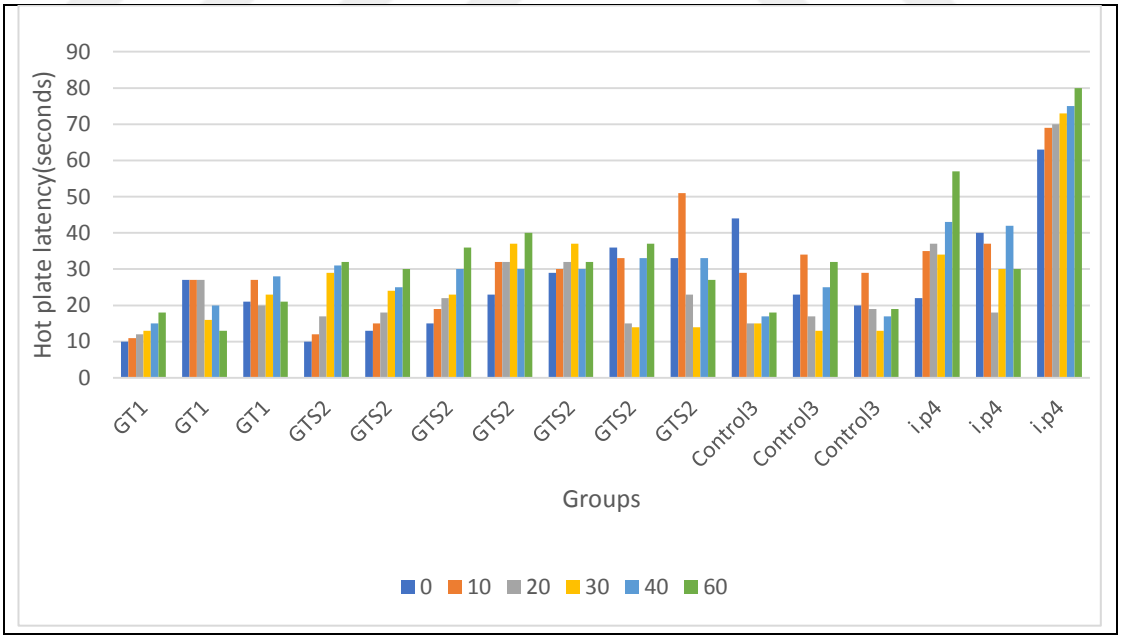


Figure 3.1. Hot plate latency versus groups on rats (tramadol 28mg/kg)

## 3.3. RESULTS OF STATISTICAL ANALYSES

### 3.3.1. Control Group (Placebo)

There was the least analgesia effect was observed on rats because it was not applied any application to placebo group. Placebo group was used for control for other groups. It was evaluated each data groups compared with to the time-dependent system. Kruskal Wallis ANOVA test was used with statistical program NCSS with all groups as shown in Table 3.1.

Table 3.1. All group data at initial time

| Group | Count | Sum of ranks | MeanRank | Z-Value | Median | P- Value |
|---|---|---|---|---|---|---|
| 1 | 3 | 17.5 | 5.83 | -1.0763 | 21 | 0.34978 |
| 2 | 7 | 53 | 7.57 | -0.688 | 23 | |
| 3 | 3 | 28.5 | 9.5 | 0.4036 | 23 | |
| 4 | 3 | 37 | 12.33 | 1.5471 | 40 | |

Rats were placed in hot plate at 0-10-20-30-40 and 60 minutes time intervals. After successfully completing the procedures, the initial latency responses were noted for each time intervals. The results are shown in the figure below Figure 3.2.

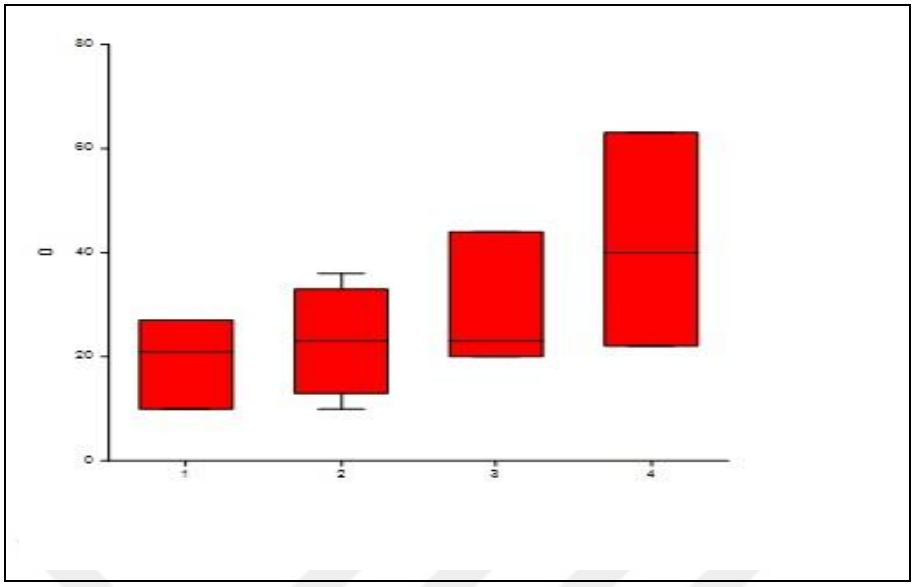


Figure 3.2. initial time sonication application to rats

### 3.3.2. 10 Minutes - Sonication Application to Rats

In this part of the experiment, the rats were treated hydrogel with tramadol (initial or without sonication) and sonication frequency at 40 kHz. Firstly, tramadol hydrogel was applied then sonication was applied to rats. It was applied every 10-20-30-40 and 60 minutes time interval and initial data (without sonication). In this application, paw licking or jumping response average occurred at 15 seconds.

After successfully completing the procedures, latency responses were noted for each time intervals as seen in Table 3.2 and Figure 3.3.

Table 3.2. All group data at 10 minutes

| Group | Count | Sum of ranks | MeanRank | Z-Value | Median | P-Value |
|---|---|---|---|---|---|---|
| 1 | 3 | 12 | 4 | -1.8162 | 27 | 0.05998 |
| 2 | 7 | 54 | 7.71 | -0.5822 | 30 | |
| 3 | 3 | 27 | 9 | 0.2018 | 29 | |
| 4 | 3 | 43 | 14.33 | 2.3544 | 37 | |

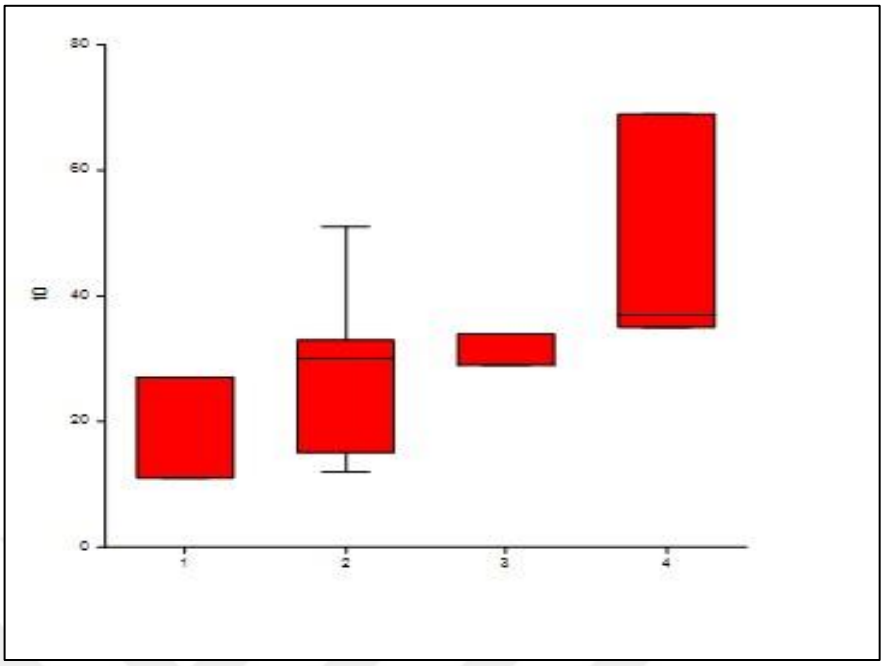


Figure 3.3. 10 minutes sonication application to rats

### 3.3.3. 20 Minutes - Sonication Application to Rats

In this part of the experiment, firstly tramadol hydrogel was applied then sonication was applied to rats. Sonication was applied to rats with ultrasonic transducer as transmitter applied with power source at 15volt. It was applied every 10-20-30-40 and 60 minutes time interval and initial data (without sonication). In this part, the paw licking or jumping response was as same as 10 minutes result.

After successfully completing the procedures, latency responses were noted for each time intervals (Table 3.3 and Figure 3.4).

Table 3.3. All group data at 20 minutes

| Group | Count | Sum of ranks | MeanRank | Z-Value | Median | P-Value |
|---|---|---|---|---|---|---|
| 1 | 3 | 22 | 7.33 | -0.4709 | 20 | |
| 2 | 7 | 61.5 | 8.79 | 0.2117 | 22 | |
| 3 | 3 | 15 | 5 | -1.4126 | 17 | 0.26754 |
| 4 | 3 | 37.5 | 12.5 | 1.6144 | 37 | |

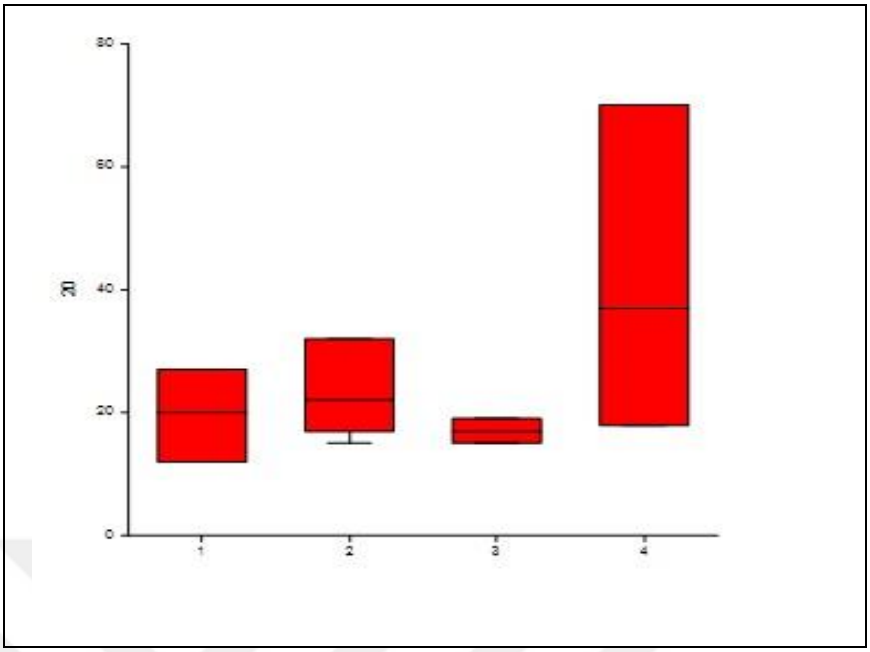


Figure 3.4. During 20 minutes sonication application to rats

### 3.3.4. 30 Minutes - Sonication Application to Rats

In this part of the experiment, the rats were treated tramadol hydrogel. Firstly, tramadol hydrogel was applied then sonication was applied to rats. Sonication was applied to rats with ultrasonic transducer as a transmitter, with power supply at 15volt. It was applied every 10-20-30-40 and 60 minutes time interval and initial data (without sonication). Paw licking or jumping response was becoming lately than 10 and 20 minutes. It was observed approximately 20 seconds.

After successfully completing the procedures, latency responses were noted for each time intervals. Their jumping or paw licking responses were observed more specifically. There is no significantly difference (Table 3.4, Table 3.5 and Figure 3.5).

Table 3.4. All groups data at 30 minutes

| Group | Count | Sum of ranks | MeanRank | Z-Value | Median | P-Value |
|---|---|---|---|---|---|---|
| **1** | **3** | **17.5** | **5.83** | **-1.0763** | **16** | |
| 2 | 7 | 67.5 | 9.64 | 0.8468 | 24 | |
| 3 | 3 | 10 | 3.33 | -2.0853 | 13 | 0.03824 |
| 4 | 3 | 41 | 13.67 | 2.0853 | 34 | |

Table 3.5. Z Test p-values at 30min.

| 30 | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| **1** | 0 | 1.1656 | 0.6465 | 2.0256 |
| **2** | 1.1656 | 0 | 1.9305 | 1.2311 |
| **3** | 0.6465 | 1.9305 | 0 | 2.672 |
| **4** | 2.0256 | 1.2311 | 2.672 | 0 |

There is a statistically significantly difference between third and forth group t = 30. If z value higher than 1.96, medians will significantly different with Regular Test. If z value higher than 2.6383, medians will significantly different with Bonferroni Test.

In short, if the P value is quite high, we can not reject the hypothesis1. Therefore, there is significant difference between different time intervals. P=0.03824 <0.05 there it was obtained significant difference.

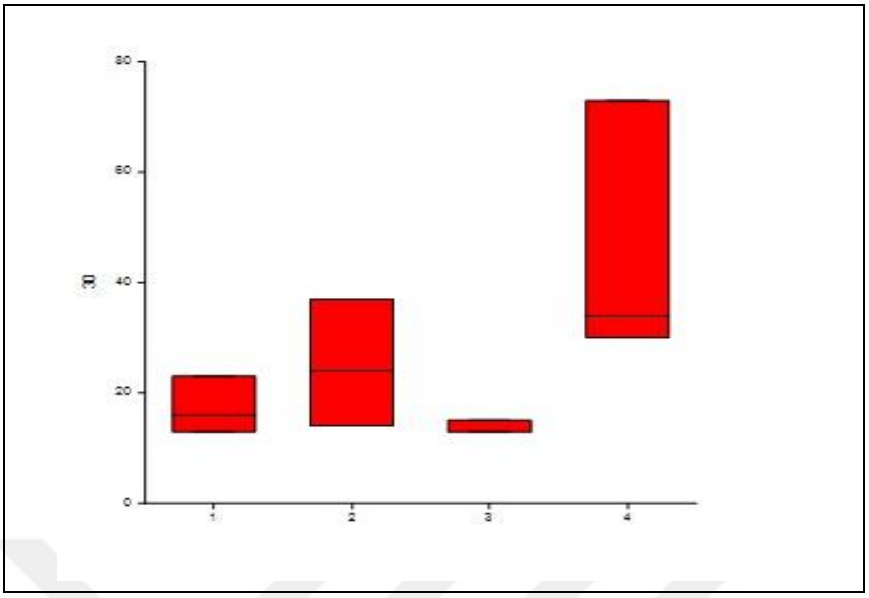


Figure 3.5. During 30 minutes sonication application to rats

### 3.3.5. 40 Minutes - Sonication Application to Rats

In this part of the experiment, firstly tramadol hydrogel was applied then sonication was applied to rats. Sonication was applied to rats with ultrasonic transducer as a transmitter, with power supply at 15 Volt. It was applied every 10-20-30-40 and 60 minutes time interval and to initial data (without sonication). It was observed at 20 seconds like 30 minutes results.

After successfully completing the procedures, latency responses were noted for each time intervals. Their jumping or paw licking responses were observed more specifically in 40 minutes (Table 3.6 and Table 3.7 and Figure 3.6).

Table 3.6. All group data at 40 minutes

| Group | Count | Sum of ranks | MeanRank | Z-Value | Median | P-value |
|---|---|---|---|---|---|---|
| 1 | 3 | 12 | 4 | -1.8162 | 20 | |
| 2 | 7 | 68.5 | 9.79 | 0.9527 | 30 | |
| 3 | 3 | 10.5 | 3.5 | -2.018 | 17 | 0.00708 |
| 4 | 3 | 45 | 15 | 2.6234 | 43 | |

Table 3.7. Z test p-values at 40min.

| 40 | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| **1** | 0 | 1.7702 | 0.1293 | 2.8444 |
| **2** | 1.7702 | 0 | 1.9232 | 1.5954 |
| **3** | 0.1293 | 1.9232 | 0 | 2.9737 |
| **4** | 2.8444 | 1.5954 | 2.9737 | 0 |

There is significantly diffence; p- value, p=0.00708 <0.05.

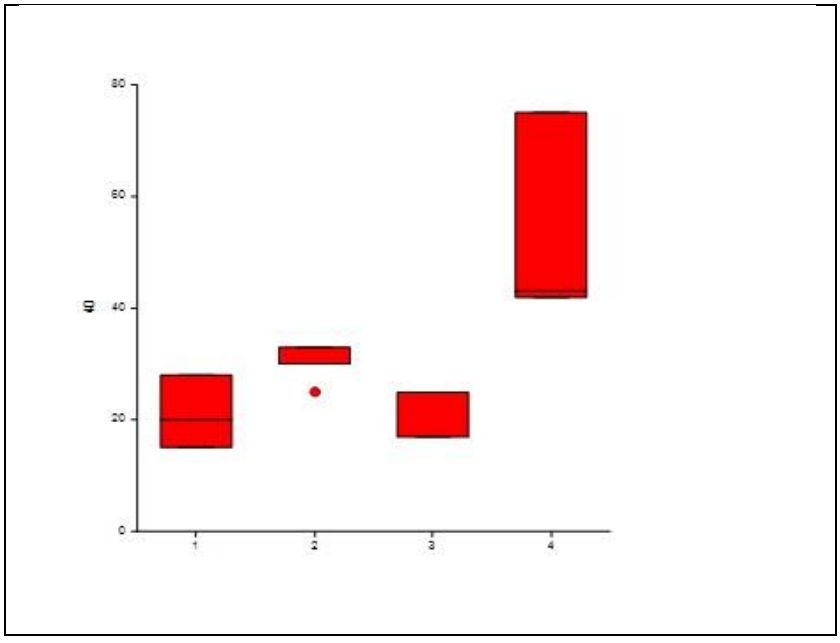


Figure 3.6. During 40 minutes sonication application to rats

### 3.3.6. 60 Minutes - Sonication Application to Rats

In this part of the experiment, firstly tramadol hydrogel was applied then sonication was applied to rats. Sonication was applied to rats with ultrasonic transducer as a transmitter, with power supply at 15volt It was applied every 10-20-30-40 and 60 minutes time interval and initial data (without sonication). It was the latest response was observed at 60 minutes application as expected. Average response result was 25 seconds because tramadol absorption occurs lately in the body.

After successfully completing the procedures, latency responses were noted for each time intervals. From 30 minutes it was observed that analgesia has started to effect rats. Their jumping or paw licking responses were observed more specifically in 60 minutes (Table 3.8 and Table 3.9 and Figure 3.7).

Table 3.8. All group data at 60 minutes

| Group | Count | Sum of ranks | MeanRank | Z-Value | Median | P-value |
|---|---|---|---|---|---|---|
| 1 | 3 | 8.5 | 2.83 | -2.2871 | 18 | |
| 2 | 7 | 72.5 | 10.36 | 1.3761 | 32 | |
| 3 | 3 | 16.5 | 5.5 | -1.2108 | 19 | 0.0294 |
| 4 | 3 | 38.5 | 12.83 | 1.7489 | 57 | |

There is significantly difference at most. There is more significant late absorption of tramadol hydrogel at 60 minutes.

Table 3.9. Z Test p-values at 60min.

| 60 | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| **1** | 0 | 2.3003 | 0.689 | 2.5839 |
| **2** | 2.3003 | 0 | 1.485 | 0.757 |
| **3** | 0.689 | 1.485 | 0 | 1.8949 |
| **4** | 2.5839 | 0.757 | 1.8949 | 0 |

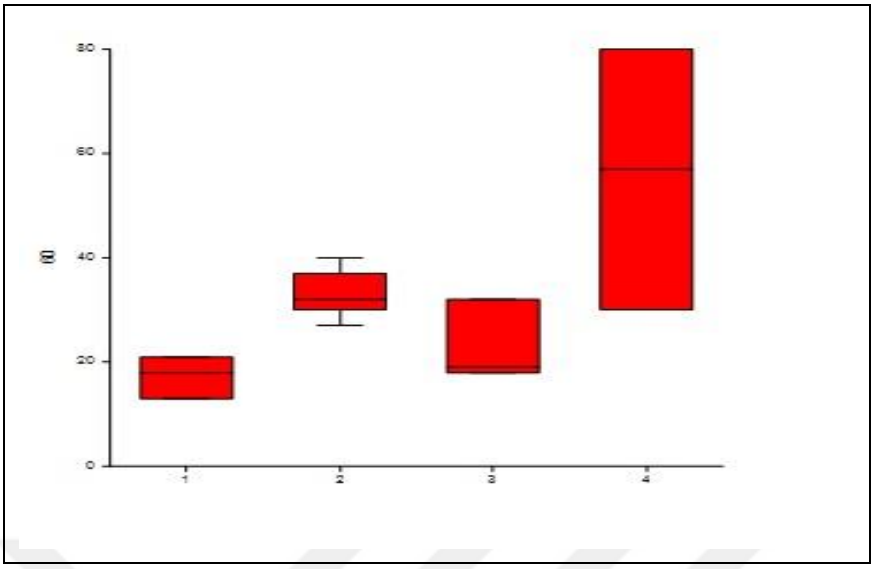


Figure 3.7. During 60 minutes sonication application to rats

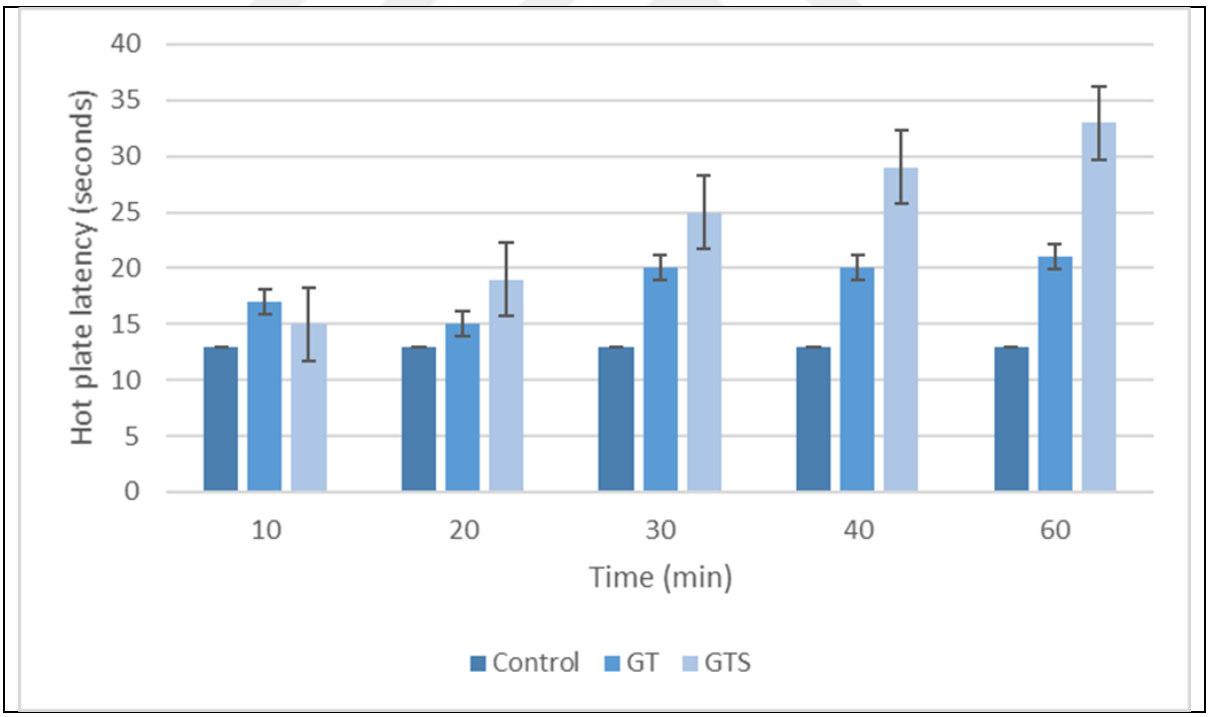


Figure 3.8. Tramadol hydrogel (28mg/kg) latency times in rats versus time (min)

# 4. DISCUSSION

Low-frequency sonophoresis has many advantages compared to oral and injection methods. Tezel et al. showed low-frequency is related to dependence of permeability enhancement on frequency and intensity[19]. Levy et al. showed that when convection and cavitation were mixed with mannitol and inulin. In this study, we used one of active transportation method. Low-frequency sonophoresis were applied with 40 kHz ultrasonic transducer.

Ultrasonic transducer was applied by hand because the bandage and baby socks rubber that we prepared for rats became the irritators for the rats. Sonophoresis application can be done with a different design in the future.

According to Blankenship et al. manuscript" it was evaluated the efficacy of four dosages. These dosages are 4, 12.5, 25 and 50 mg tramadol per kg body weight and 3 routes of administration (per oral, i.p, subcutaneous) using hot plate and tail flick test. The i.p. route of administration was also effective at dosages of 12.5 mg, 25 mg and 50 mg tramadol per kg body weight, though sedation was observed at dosages of 25 mg and 50 mg per kg body weight" [20].

The study demonstrated that at 60 minutes after i.p. administration of 28 mg tramadol per kg body weight, rats had a prolonged latency response to a heat source. Though greater dosages would be applied, it is also caused sedation time longer. Tramadol has an alternative to other opioids, as has been showed in both acute and chronic pain syndromes. It has no clinically associated effects on respiration, is related with a low incidence of constipation, demonstrates positive effects on immune system function, has a low potential for addiction. Tramadol is also free of the prostaglandin effects associated with nonsteroidal anti-inflammatory drugs, which may lead to gastrointestinal bleeding, renal impairment, bronchospasm and reduced platelet activity. The i.p. injection route permits administration of large volumes of drugs into the abdominal cavity that are rapidly absorbed.

In addition to, Spraque Dawley rats that received 28 mg tramadol per kilogram body weight i.p. and transdermal applications with sonication had behavioral responses to

tramadol that included minimized responsiveness to tactile stimuli and decreased cage activity [21].

The method of injection and oral medications, which have become phobias as it is known by people, unlike under the skin of active ingredients enables this sonication device to be sent.

In this study, the model drug can be changed in the future. Tramadol hydrogel data can be compared with another narcotic analgesic drug. Another option is that the active transport method of drug release can be changed.

A major finding of our study was that receiving dosages of 28 mg tramadol per kilogram body weight i.p. and transdermal applications had impaired motor function.

# 5. CONCLUSION

Low-frequency sonophoresis provides to increase the permeability of skin through the process of acoustic cavitation above the skin. It causes the formation of acoustic micojects on the surface of the skin. Low-frequency sonophoresis showed to let transdermal delivery of tramadol hydrogel.

In this study, it was used the hot plate latency test to compare latency in response to acute thermal pain after transdermal tramadol application with sonication (40kHz). This study investigated the possibility of developing transdermal tramadol with sonication application allowing fast analgesic effect of tramadol in a 40-60 minutes. It was found that there is a significant difference ($p<0.05$) between 28mg/kg transdermal tramadol hydrogel alone and transdermal tramadol hydrogel with sonication as from 40 and 60 minutes application. The data were all expressed as means ± SEM., n=3. Z-Value Testing which is called Kruskal Wallis Test was used to examine the statistical significance of the difference between groups.

At this study, it was found that when administering 40-60 minutes sonication was found effective. The maximum latency in response to acute thermal pain that was observed after 60 minutes. The bioavailability of the transdermal hydrogel with sonication was increased almost two and three times (respectively after 40 and 60 minutes) more than transdermal hydrogel application.

It was observed no effect on latency in response to acute thermal pain in any of the rats that were given transdermal tramadol as hydrogel with sonication application at initial, 10, 20 and 30 minutes later. So, it was calculated that the transdermal as hydrogel with sonication application did not seem to effective until 40 minutes.

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# APPENDIX A: ETHICAL APPROVAL FORM

YEDİTEPE ÜNİVERSİTESİ

T.C. YEDİTEPE ÜNİVERSİTESİ, DENEY HAYVANLARI ETİK KURULU

(YÜDHEK)

ETİK KURUL KARARI

| Toplantı Tarihi | Karar No | İlgi | Proje Yürütücüsü |
|---|---|---|---|
| 01.06.2018 | 674 | 29.05.2018 | Dr. Öğretim Üyesi: Gülengül Duman |

'Transdermal yolla uygulanan [6] Tramadol hidrojelin' ultrason yöntemiyle deriden emiliminin arttırılmasının sıçanlar üzerinde araştırılması' adlı bilimsel çalışma etik kurulumuzda görüşülmüş olup, çalışmanın etik kurallara uygun olduğuna oy birliğiyle karar verilmiştir.

| Etik Onay Geçerlilik Süresi: 3 Yıl | Hayvan Türü ve cinsiyeti: Sıçan 8 | Hayvan Sayısı: 21 |
|---|---|---|

| GÖREVİ | ADI SOYADI | |
|---|---|---|
| Başkan | Prof. Dr. Bayram YILMAZ | |
| Başkan Yardımcısı | Prof. Dr. Erdem YEŞİLADA | KATILMADl |
| Raportör | Vet. Hekim Engin SÜMER | |
| Üye | Prof. Dr. M. Ece GENÇ | KATILMADI |
| Üye | Prof. Dr. Rukset ATTAR | |
| Üye | Doç. Dr. Soner DOĞAN | |
| Üye | Doç. Dr. Ediz DENİZ | |
| Üye | Prof. Dr. Gamze TORUN KÖSE | |
| Üye | Doç. Dr. Aylin YABA UÇAR | |
| Üye | Hakan GÖKSEL | |
| Üye | Ahmet ŞENKARDEŞLER | |

Figure A.1. Ethical Approval Form