ÇUKUROVA UNIVERSITY
INSTITUTE OF NATURAL AND APPLIED SCIENCES

MSc THESIS

Rumooz SHAYYAH

DESIGNING AND IN VIVO EVALUATION OF MICROEMULSION FORMULATION CONTAINING CAPSAICIN FOR THE TREATMENT OF NEUROPATHIC PAIN

DEPARTMENT OF BIOTECHNOLOGY

ADANA-2018

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We certify that the thesis titled above was reviewed and approved for the award of degree of the Master of Science by the board of jury on 09/07/2021

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This MSc Thesis is written at the Department of Biotechnology, Institute of Natural and Applied Sciences of Çukurova University.
Registration Number: 5902

T.C. ÇUKUROVA ÜNİVERSİTESİ FEN BİLİMLERİ ENSTİTÜSÜ MÜDÜRLÜĞÜ 1923

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This thesis was supported by the Scientific Research Project Unit of Çukurova University with a project number of TYL-2019-12066

ABSTRACT

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DESIGNING AND IN VIVO EVALUATION OF MICROEMULSION FORMULATION CONTAINING CAPSAICIN FOR THE TREATMENT OF NEUROPATHIC PAIN

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Year: 2021, Pages: 69
Jury : Prof. Dr. Fazilet AKSU
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Capsaicin (CAP) is the compound found in chili peppers and responsible for their bitter taste, burning, and irritant effect. It has been used for the treatment of pain disorders, especially in neuropathic pain (NP). CAP is more effective at higher doses however, its side effects increase with higher doses. In this study, novel oral microemulsion capsaicin formulation (ME CAP) has been developed in order to increase the solubility, in vivo bioavailability and decrease adverse effects of CAP. The effect of ME CAP evaluated by comparing with commercially available oral CAP preparations on neuropathic mice. Micro emulsions are dispersions of water and oil. This form of oil-water mixtures is homogeneous, transparent, and thermodynamically stable. Stabilization of mixture is achieved by a surfactant, usually in combination with a co-surfactant. The micro-emulsion was prepared using oleic acid, Tween 80, propylene glycol, ethanol and water. Pseudo ternary phase diagrams were constructed to determine the area of the microemulsion at different ratios of surfactant to co-surfactant. The optimum formulation on different physicochemical characteristics such as globule size, pH, and stability were studied.

The sciatic nerve partial tight ligation (PSL) model was used to induce NP in mice. Thermal and mechanical stimuli were used to evaluate allodynia, which is one of the most important signs of NP. After two weeks, NP was tested using the cold plate method and von Frey filaments testing. The mice in the treatment group were administered 10 mg/kg capsaicin by oral gavage. The effects of conventional (classic) capsaicin, which is frequently used in neuropathies, and ME CAP in this study were compared. Our results showed that the presented formulation is more effective than classic CAP with Von Frey test while showing no difference between them with the cold plate test. Therefore, the developed ME CAP formulation at lower doses could reduce side effects and improve the bioavailability of the orally administered CAP in the treatment of NP, and thus would get a good patient compliance.

Key words: Capsaicin, neuropathic pain, microemulsion, mice

ÖZ

YÜKSEK LİSANS TEZİ

NÖROPATİK AĞRI TEDAVİSİ İÇİN KAPSAisin İÇEREN MİKROEMÜLSİYON FORMÜLASYONUNUN TASARIMI VE İN VIVO DEĞERLENDİRİLMESİ

Rumooz SHAYYAH

ÇUKUROVA ÜNİVERSİTESİ
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Kapsaisin (CAP), ağrı ve inflamatuar hastalıkların tedavisi için klinikte kullanılmaktadır. CAP, oda sıcaklığında suda zor çözünen, yağda çözünen bir bileşiktir. Düşük çözünürlüklü olması, sulu çözelti içinde iyi dağılmasını zorlaştırır ve etkili salınımı için bir engel oluşturur. Bu çalışma, ağrı ve inflamasyona karşı kapsaisinin terapötik olarak daha etkili bir mikroemülsiyon formülasyonu (ME CAP) hazırlamak için planlanmıştır. Mikroemülsiyonlar homojen, şeffaf, termodinamik olarak stabil su ve yağ dispersiyonlarıdır, genellikle bir yardımcı yüzey aktif madde (tipik olarak kısa zincirli bir alkol) ile kombinasyon halinde bir yüzey aktif cismi ile stabilize edilir. Mikroemülsiyon, oleik asit, Tween 80, propilen glikol, etanol ve su kullanılarak hazırlanmıştır. Psödoterner faz diyagramları, farklı yüzey aktif madde ve ortak yüzey aktif madde oranlarında mikroemülsiyonun alanını belirlemek için oluşturulmuştur. Globül boyutu, pH ve stabilite gibi farklı fizikokimyasal özellikler üzerine optimum formülasyon çalışılmıştır.

Çalışmada deneysel nöropatik ağrı oluşturmak için fare siyatik sinir kısmi sıkı ligasyon (PSL) modeli kullanıldı. Nöropatik ağrının en önemli belirtilerinden biri olan allodini'yi değerlendirmek için termal ve mekanik uyaranlar kullanıldı. İki hafta sonra, nöropatik ağrı, soğuk plaka (termal allodini için) ve Von Frey filament (mekanik allodini için) testleri kullanılarak değerlendirildi. Tedavi grubundaki farelere oral gavaj yoluyla 10 mg/kg kapsaisin uygulandı. Klasik CAP ve ME CAP uygulanan guruplarda etkileri karşılaştırıldı. Çalışmanın sonuçları, hazırlanan formülasyonun (ME CAP) Von Frey testinde klasik CAP'den daha etkili olduğunu ancak soğuk plaka testinde farklı olmadığını göstermiştir. Bu sonuca göre hazırlanan formülasyonun mekanik allodininin belirgin olduğu NP ağrı tedavisinde klasik oral formülasyondan daha etkili olabileceği ve daha düşük dozda daha az yan etkiyle daha fazla hasta uyumu sağlanabileceği söylenebilir.

Anahtar Kelimeler: Kapsaisin ,nöropatik ağrı, microemülsiyon, fare

EXPANDED ABSTRACT

Capsaicin (CAP) is the compound found in chili peppers and responsible for their hot pungent (bitter) taste, burning, and irritant effect when it comes into contact . It was isolated in 1846 and its structure determined in 1919. However, it has received increased scientific attention in recent years based on its potential analgesic activity. CAP was very important to understand TRPV1 channels and physiological and pathological processes. It has been used in different clinical conditions and in the treatment of pain disorders, especially in neuropathic pain (NP). Moreover, CAP has effects on the respiratory, gastrointestinal, cardiovascular, limbic and, thermoregulatory systems, which they differ depending on the dose and application rate. CAP is more effective at higher doses but its side effects increase with higher doses.

In this study, novel oral microemulsion capsaicin formulation (ME CAP) has been developed in order to increase the solubility, in vivo bioavailability and decrease adverse effects of CAP. The effect of ME CAP evaluated by comparing with commercially available oral CAP preparations (classic capsaicin) on neuropathic mice.

ME CAP was prepared using the oleic acid, Tween 80, propylene glycol, ethanol and water. Pseudo ternary phase diagrams were constructed to determine the area of the microemulsion at different ratios of surfactant to co-surfactant. The optimum formulation on different physicochemical characteristics such as globule size, pH, and stability were studied. Thereafter, the sciatic nerve partial tight ligation (PSL) model (Seltzer's model) was used to induce NP in mice. For anesthesia, ketamine and xylazine were administered intramuscularly (IM). Under aseptic conditions, an incision of approximately 1 cm was applied to the biceps femoris muscle where was dissected to reach the mouse right leg sciatic nerve. After this procedure, the sciatic nerve was removed from the underlying tissues, stretched out of the body with beveled forceps, and knotted with 4.0 non-

absorbable, silk thread. Finally the incision was closed with the same type of thread.

After injury, animals develop protection behavior and licking of the affected limb, suggesting spontaneous pain. Other behavioral changes are also reported in this model one week after surgery and lasting for approximately six weeks. Animals were tested pre-surgery and 1-2 weeks post-surgery.

The mice were divided into six groups according to the experimental protocol. The first treatment group was ME CAP group, in which NP was induced and after 14 days of surgery 10 mg/kg novel ME CAP were administered by oral gavage. The second treatment group was classic CAP group, in which NP was induced and 14 days after surgery 10 mg/kg classic CAP were administered by oral gavage. The ME capsaicin-treated mice group and the corresponding other groups were tested at two hours post oral gavage using Cold Plate (CP) and Von Frey (VF) tests.

Thermal and mechanical stimuli were used to evaluate allodynia, which is one of the most important signs of NP. The CP device consists of a circular metal plate that is arranged with a transparent cylindrical plastic glass and it can be fixed at 5 °C. It tests the responses of untethered mice to low temperature stimulation of the plantar side of the paw and it used for evaluating thermal allodynia. The VF filaments (or hairs) are plastic filaments, with a length of 5 cm and various diameters, fixed on applicators, component of 20 monofilaments, from 0.008 grams to 300 grams. Their end is not sharp but blunt. Rodents exhibit a paw withdrawal reflex when the paw is touched unexpectedly. VF filaments are applied to the plantar surface of each hind paw with a series of ascending forces. With our procedure in mice, for evaluating mechanical allodynia, the most commonly used filaments are 0.16, 0.4, 0.6, 1, 1.4, 2, 4, 6 and 8 g.

In this study we observed that ME CAP was transparent, thermodynamically stable dispersions. According to allodynia test results no

significant difference was seen in CP test where a significant difference was observed in the ME CAP compared with Classic CAP in VF test.

This study showed that the ME CAP was more effective on mice than classic CAP in mechanical allodynia but not in thermal allodynia. Therefore, the novel microemulsion formulation at lower doses could reduce side effects and improve the bioavailability of the oral administration of CAP in the treatment of NP which shows mechanical sensitivity, and thus would get a good patient compliance.

ACKNOWLEDGEMENT

First of all, I must be thankful to Allah for finishing the research and to my great supervisor, Prof. Dr. Fazilet AKSU, who continuously guided me throughout every step of my study and generously shared her time and knowledge with me. She has been my inspiration as I hurdle all the obstacles in the completion of this research work.

I also need to thank Dr. Öğr. Üyesi Umay Merve GÜVEN for her encouragement, motivation, enthusiasm, immense knowledge, guidance and support from the initial to the final level enabled me to develop an understanding of this research. My special thanks to Dr. Ares ALİZADE for his guiding collaboration, training and friendly helps. My particular thanks must be extended to all academic and technical staff members of Pharmacology, Pharmaceutical Technology, and Biotechnology Departments at Cukurova University.

I also need to thank my beloved father Mohammad Sabit, my beloved mother Faiza, my brothers Mohammad Kamel and Hasan and my fiancé Amr also has a special thank you. All the support they have provided me over the years was the greatest gift anyone has ever given me. Also, I am heartily thankful.

Lastly, I would like to acknowledge all my close friends for usual support and cooperation that will never be forgotten.

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LIST OF SYMBOLS AND ABBREVIATONS

CAP	: Capsaicin
NP	: Neuropathic pain
ME CAP	: Novel oral microemulsion capsaicin
PSL	: Partial sciatic nerve tight ligation
SEDDS	: Self-emulsifying drug delivery systems
CNS	: Central nervous system
O/W	: Oil-in-water
W/O	: Water-in-oil
IASP	: International Association for the Study of Pain
ATP	: Adenosine triphosphate
NGF	: Nerve growth factor
GDNF	: Glial cell derived neurotropic factor
TENS	: Transcutaneous electrical nerve stimulation
PNS	: Peripheral nerve stimulation
NRS	: Nerve root stimulation
SCS	: Spinal cord stimulation
DBS	: Deep brain stimulation
MCS	: Epidural motor cortex stimulation
rTMS	: Repetitive transcranial magnetic stimulation
TCAs	: Tricyclic antidepressants
SNRI	: Serotonin and norepinephrine reuptake inhibitors
PHN	: Postherpetic neuralgia
TRPV1	: Transient Receptor Potential V1 receptor
ME	: Microemulsion
HLB	: Hydrophilic-lipophilic balance
PG	: Propylene glycol
PEG	: Polyethylene glycol

S	: Surfactant
CoS	: Co-surfactant
PDI	: Polydispersity index
IM	: Intramuscular
CP	: Cold Plate
CPL	: Cold Plate Latency
VF:	: Von Frey
VFTH	: Von Frey Thresholds

1. INTRODUCTION

Neuropathic pain (NP) is a chronic pain associated with damage to a part of the peripheral or central nervous system (CNS), impaired function, or altered excitability. Treatment of NP is difficult and known analgesics do not respond this type of pain. Apart from analgesic drugs, good results may not always be obtained in treatment with other drugs such as tricyclic antidepressants or local anesthetics. Recently, the use of capsaicin, which founds in chili peppers in NP, has gained interest. However, capsaicin needs to be used at high doses to be effective but severe side effects occur at high doses (Üçeyler and Sommer 2014).

The effect of capsaicin in NP is dose-dependent. It is more effective at higher doses however, its side effects increase with higher doses. Capsaicin is mostly used as topical preparations. However, when using in topical preparations capsaicin causes irritation and a burning sensation. It enhances sensitivity to noxious stimuli, and causes capsaicin-induced dermal pain [Bode and Dong 2011]. In the chronic treatment of many diseases, drugs are mostly used by orally. Almost forty percent of new drug candidates slightly soluble in water, so their oral bioavailability is also low. Since hydrophobic drugs are low in water solubility, new formulations are being prepared to enhance the oral absorption of these drugs. “Self-emulsifying drug delivery systems (SEDDS)” are used in the design of these drugs’ formulations (Gursoy and Benita 2004). The aim of the development of these formulations is to prepare new drugs that show the desired pharmacological effect at lower doses and have reduced side effects.

Studies that increase the bioavailability and the safety index of capsaicin are important to obtain preparations with low doses, reduced side effects and desired pharmacological effects.

In this study, novel oral capsaicin formulation has been developed as an alternative to the commercially preparations containing capsaicin in NP. Oil-in-water microemulsions of capsaicin by using oleic acid as an oil phase, Tween® 80

as a surfactant, Propylene glycol and Ethanol as cosurfactants and water were prepared. Furthermore, oral preparations developed using nanotechnological methods are more advantageous than classical oral preparations in terms of effect, absorption, and reaching the site of action.

Therefore, in this study, we aimed to develop an orally effective delivery system, oil-in-water (O/W) microemulsions, to increase the solubility, *in vivo* bioavailability and decrease adverse effects of capsaicin. The aim of the development of this formulation is to prepare a new capsaicin preparation that show the desired pharmacological effect with lower doses and have reduced adverse effects. The effect of developed formulations of capsaicin on mice with experimental neuropathy was evaluated by comparing with commercially available oral capsaicin preparations.

2. LITERATURE REVIEW

The term “pain” derives its origin from the Latin word “poena” (punishment, torture). According to the definition made by the “International Association for the Study of Pain (IASP)” in 1979, “pain is an unpleasant sensory and emotional experience that accompanies or can be identified by existing or possible tissue damage”. In this definition, it is evident that the subjective, emotional and psychological aspects of pain are combined. For these reasons, the response to a painful stimulus varies from person to person, even in the same person may be different (Kumar and Elavarasi 2016; Mayer et al. 1999).

Pain is occurring when an intense (noxious, painful) stimulus, which can be a threat to normal tissue, activates nociceptors. Depending on the responsible stimulus pain may range from sharp, prickling, or shock-like to dull, aching, or burning. Sharp pain results from the activation of nociceptors by myelinated Aδ nerve fibers, while dull, aching pain results from activation of nociceptors by unmyelinated C fibers (specific nerve cells in the body that detect noxious stimuli or changes that could have damaged the body, such as extreme heat or cold, pressure, pinching, and chemicals).

2.1. Classification of pain

2.1.1. According to the duration of the pain

2.1.1.1. Acute pain

Acute pain begins suddenly, has a close relationship with the lesion that causes it in terms of place, time, severity, starts with tissue damage, gradually decreases and disappears during recovery. It is sharp in quality and probable limited duration. It disappears when there is no longer an underlying reason for the pain (Macintyre et al. 2010).

2.1.1.2. Chronic pain

Chronic pain can last for years and range from mild to severe even after the original injury has healed and often there could not be any clearly definably cause (Macintyre et al. 2010) or in broad categories based on the origin of the injury or pain fibers (Johnson, Borsheski, and Reeves-Viets 2013). It persists at intervals of months or even years, much longer than the usual duration of acute painful diseases or the recovery time of damage.

2.1.2. According to the Region of Origin

2.1.2.1. Somatic pain

It occurs as a result of stimulation of pain receptors in the peripheral tissues such as skin, muscle and joints and is localized the source (Johnson, Borsheski, and Reeves-Viets 2013). Somatic pain is more often a pain carried by somatic nerve fibers. It starts suddenly, is sharp, well localized, in a stinging, whining, throbbing style. It is detected in the spreading region of the nerves. Pain usually seen in cases such as trauma, fracture, and dislocation is called somatic pain (Mayer et al. 1999).

2.1.2.2. Visceral pain

It can be seen depending on damage or injuries to the internal organs such as chest, abdomen, and pelvis. It is not well localized and frequently the pain is referred to another area of the body (Johnson, Borsheski, and Reeves-Viets 2013). Visceral pain is a type of acute pain, which, occurs due to the disease or abnormal function of the internal organs or membranes such as parietal pericardium and peritoneum. Visceral pain starts slowly, it is blunt and whining and it is difficult to localize (Mayer et al. 1999).

2.1.2.3. Sympathetic pain

Sympathetic pain caused by sympathetic nervous system activation. Vascular origin pains, reflex sympathetic dystrophy are some examples of sympathetic pain. These pains are in burning style (Mayer et al. 1999).

2.1.3. According to the Mechanisms

2.1.3.1. Deafferantation Pain

Deafferantation pain is the pain caused by the interruption of the transmission of sensory stimuli to the central nervous system due to lesions in the central or peripheral central nervous system (Mayer et al. 1999).

2.1.3.2. Reactive Pain

It is seen by stimulation of pain receptors by reflex activation of motor or sympathetic afferent nerves. Myofacial pain can be given as an example of this type of pain (Önal SA 2004, and Raj PP 2002).

2.1.3.3. Psychosomatic pain

It is the expression of the patient's psychic or psychosocial problems in the form of pain. An example of this is the clinical condition we call somatization. The patient uses pain in a sense, expresses various personal, economic and social problems in the form of pain, trying to attract attention and gather the attention of society on itself (Hansson 2002).

2.1.3.4. Nociceptive pain

Nociceptive pain occurs due to the fact that a number of physiopathological events and processes stimulate pain sensors, which we call nociceptor (Hansson 2002).

Nociceptive pain acts as a significant alarm system such pain can warn us about internal problems in our body, from cardiac ischemia to a burst appendix (Prescott and Ratté 2017).

It continues only in the maintained presence of noxious stimuli. Certain diseases could generate frequent or ongoing noxious stimuli to produce chronic nociceptive pain. One example is osteoarthritis, where changes in the joint can allow normal weight-bearing to produce forces sufficient to activate nociceptors which can also be found in the internal organs, including muscle and viscera, although their alarm signals are less easy to locate and may not always be consciously felt (Torres et al. 2006).

2.1.3.5. Neuropathic pain

Neuropathic pain results from disease conditions or trauma to the nerves. It is a type of pain in the peripheral nerves caused by the direct influence of nociceptors as a result of trauma or a metabolic disease (Hansson 2002). The process and mechanisms of formation are mentioned in more detail in the following section.

2.1.4. Neuropathic pain

Neuropathic pain happens with damage to a peripheral nerve, dorsal root, or any place in the CNS. Due to the nerve itself is injured, NP can continue to be abnormally activated, creating a hyper-excitable state within the CNS (Johnson, Borsheski, and Reeves-Viets 2013). This pain seems to go out of nowhere, rather than in response to any specified injury. NP can be stated as a burning, freezing, stabbing and, shooting pain found along the distribution of that nerve. The formation process, diagnosis and mechanisms are explained in more detail in the next section. The incidence of NP is reported at 7-10 percent (Van Hecke et al. 2014).

NP usually occurs long after nerve damage, as opposed to inflammatory pain, as a result of dysfunction or primary lesion of the nervous system. A lesion or disease of the somatosensory system, including peripheral fibers Aβ, Aδ and, C fibers and, central neurons lead to NP. The most important difference of neuropathic pain mechanisms from nociceptive pain is the role of central and peripheral mechanisms. Neuropathic pain can occur as spontaneous pain, independent of the stimulus, and can also occur after hypersensitivity or depending on the stimulus after the change of sensorial neurons (Vega et al. 2003). NP results from lesions to the PNS caused by mechanical trauma, metabolic diseases, neurotoxic chemicals, infection, or tumor invasion and involve multiple pathophysiological changes both within the PNS and in the CNS (Van Hecke, 2014). Central NP most common results from spinal cord injury, stroke, or multiple sclerosis (Ducreux et al. 2006).

NP usually occurs for a long time after nerve damage, unlike inflammatory pain. NP pain is difficult to treat, it puts serious burden on the patient, physician and economy (loss of workforce, cost of treatment, etc.) and also causes co-morbid diseases such as sleep disturbances, anxiety and depression (Woolf and Mannion 1999). NP is characterized by increased spontaneous pain without external stimulus and/or in response to a normally harmless stimulus In contrast to physiological pain. There is abnormal stimulation of the somatosensory system. NP passes through normal nociceptive pathways and is often initiated through direct tissue damage.

The mechanism underlying neuropathic pain is not fully understood, but it is complex, multifactorial, and develops over time (Vega et al. 2003; Frossard, Freund, and Advenier 2004; Shooter 2001). Damage to the central nervous system for a wide range of reasons, including toxins, infections, viruses, metabolic diseases, nutritional deficiencies, ischemia, trauma and stroke, leads to neuropathic pain. Current studies reveal that neuropathic pain is caused by cellular changes in the peripheral and central nervous system that result in sensitivity to the

transmission of painful signals. The ability of neurons to change their structure and function due to nociceptive impulses in the peripheral and central nervous system is defined as neuroplasticity. In the clinic, these changes are associated with symptoms of neuropathic pain (Frossard, Freund, and Advenier 2004).

NP conditions include autoimmune disease (e.g., multiple sclerosis), metabolic diseases (e.g., diabetic neuropathy), infection (e.g., shingles and the sequel, postherpetic neuralgia), vascular disease (stroke), trauma, and cancer (Campbell and Meyer 2006).

The most important difference of NP mechanisms from nociceptive pain is the role of central and peripheral mechanisms. NP may occur as a spontaneous pain independent of stimulus, or may occur after hypersensitivity or after a change in sensory neurons. The mechanism underlying NP is not fully understood, but it is complex, multifactorial, and develops over time.

Both stimulus-independent (spontaneous) and stimulus-dependent (evoked) pain and other symptoms such as tingling are the “positive” symptoms of NP. The “positive” symptoms of NP conditions include both stimulus-independent (spontaneous) and stimulus-dependent (evoked) pain and other symptoms such as tingling (i.e., paresthesia) (Gilron et al. 2006). The “negative” symptoms of NP are numbness, weakness, and loss of deep tendon reflexes in the involved nerve area. NP could follow different temporal profiles (e.g., continuous vs. intermittent) and could be defined with different descriptors of pain quality (Haanpää et al. 2011). Stimulus evoked pain includes allodynia, defined as pain resulting from a nonpainful stimulus (e.g., light touch of the skin), and hyperalgesia, defined as increased sensitivity to pain in response to normally painful stimulus. It is observed that these sensory abnormalities often extend beyond dermatomal or nerve territory distributions and lead to the inappropriate diagnosis of a functional or psychosomatic disorder (Gilron, Baron, and Jensen 2015).

In other words, positive symptoms are usually changed or painful sensations such as tingling, prickling, or pain described as shooting, stabbing,

burning, or electrical-like sensations. However, negative symptoms are described as decreased sensations due to loss of sensory function (Zilliox 2017).

Even the efficacy of morphine and other opioid drugs, which we can consider as the strongest analgesics, in NP is controversial today. It is accepted that these drugs can only be effective at high doses where serious side effects occur clearly (Watson 2000). For these reasons, new and effective drugs are needed for NP treatment. Since NP is partially or completely unresponsive to primary analgesic treatments (non-steroidal inflammatory analgesics and opioids), adjuvant analgesics such as tricyclic antidepressants, local anesthetics, antiepileptic drugs, gaba-mimetics and antiarrhythmics (non-primary analgesics) are widely used in medical treatment (Orza, Boswell, and Rosenberg 2000). In recent years, capsaicin has gained prominence among the drugs used in the treatment of NP (Peppin and Pappagallo 2014).

2.1.5. Mechanisms of Neuropathic Pain

2.1.5.1. Peripheral mechanisms

2.1.5.1.(1). Pathological Sensitization and Ectopic Activity

Pathological sensitization and ectopic signals occur in primary afferent nociceptive neurons after nerve damage. The sensation of pain normally leads to the activation of C fibers and Aδ fibers of primary afferent neurons. Information on the contribution of Aδ fibers to NP is limited (Frossard, Freund, and Advenier 2004). In the absence of stimulus, C nociceptors are silent but respond to painful stimuli. After nerve damage, C nociceptors become highly sensitive and develop pathological spontaneous activities (Frossard, Freund, and Advenier 2004; Rankin, Guy, and Mearow 2005). When peripheral nerve damage occurs; In addition to sensitization in peripheral nerve terminations, spontaneous ectopic activity also occurs in the area next to the dorsal root ganglion far from the injury site. These pathological changes underlie molecular and cellular changes in primary afferent nociceptors triggered by nerve damage.

Ectopic spontaneous activity following nerve damage results from increased messenger ribonucleic acid (mRNA) release to voltage-gated Na^+ channels in primary afferent neurons (Mayer et al. 1999). Concentration of sodium channels in ectopic signal regions is thought to cause hyperactivity by lowering the action potential threshold. After peripheral nerve damage, Na + channel clustering occurs not only in the injury site but also in the dorsal root ganglia proximally (Smith and Sang 2002). Spontaneous burning pain and electric shock-like sensations are observed in phantom pain seen in people with limb amputation. Therefore, sodium channel blockers provide significant benefits in the treatment of NP (Hansson 2002).

2.1.5.1.(2). Inflammation

Inflammation in the peripheral nerve trunk is one of the possible mechanisms after peripheral nerve injury. The connective bond tissue surrounding the peripheral nerves is stimulated by sensory fibers (nervi nervorum). Nervi nervorums enter the connective bond tissue of peripheral nerves through nourishing blood vessels. Proliferation and activation of macrophages in endonural blood vessels has been demonstrated in dorsal root ganglia after nerve incision and experimentally damaged nerves (Vega et al. 2003).

Nervi nervorum is a potential source of diseases of peripheral nerve diseases with an inflammatory component. Direct tissue injury at the nerve lesion site causes the release of adenosine triphosphate (ATP) and, protons from damaged cells. Cytokines (IL1, IL6, TNFα) and growth factors (NGF and leukemia inhibitory factor) are released from the endonural macrophages. Bradykinin and serotonin are released from macrophage, lymphocyte and mast cells. The primary afferent nociceptor itself also contributes to the inflammatory response through neuropeptide release, such as substance-P and calcitonin gene-associated peptide (CGRP). Serotonin causes vasodilation and edema. It activates bradykinin C fibers, phospholipase A2 in arachidonic acid metabolism and cyclooxygenase. As a result,

synthesis of prostaglandins and leukotrienes increases. Prostaglandins cause substance P secretion from sensitized nerve endings. Prostaglandins and serotonin together cause increased vascular permeability. Thus, bradykinin release is increased. Increasing bradykinin increases the production of prostaglandin and substance P. As a result, it sensitizes nociceptors in adjacent damaged areas, and hyperalgesia occurs. In other words, the low intensity stimulus is perceived as more intense. This is called peripheral sensitization and the phenomenon is called primary hyperalgesia.

Peripheral nerves damage causes up-regulation of many receptor proteins located in primary afferents neurons also. Vanilloid receptors are dominantly situated at the peripheral terminals of Aδ and C fibers and, capsaicin activates these receptors. These receptors perceive noxious heat (more than 43°C) physiologically. This condition changes dramatically after partial nerve damage and in streptozocin-induced diabetic rats. Vanilloid receptors also appear in undamaged a beta and C fibers (Lineberry and Vierck 1975).

Substance P binds to the Neurokinin-1 receptors of nociceptive neurons in the dorsal horn after released from the central terminals of Aδ and C fibers. Expression of neurokinin-1 increases from the dorsal horn after nerve damage (Frossard, Freund, and Advenier 2004).

2.1.5.1.(3). Phenotypic Change

The characteristic genes in differentiated neurons encode the functions of the neurons. These genes in primary sensory neurons are involved in transduction, conduction and synaptic transmission. An alteration (increasing or decreasing) in the regulation of hundreds of genes occurs after peripheral nerve damage (Rankin, Guy, and Mearow 2005). As a result of these alterations, changes occur in the excitability in the transduction and transmission properties of the neurons. As a result, the phenotypic structure of the neurons also changes. For example,

substance P is only released from C fibers, but also from A fibers after peripheral nerve damage.

2.1.5.1.(4). Primary Sensorial Degeneration

Peripheral nerve damage causes dorsal root ganglia neurons to disrupt the connection of cell bodies with their peripheral targets. These targets are sources of nerve growth factor (NGF) or glial cell derived neurotropic factor (GDNF). Weeks after peripheral nerve injury, atrophic changes occur in the damaged neuron. These are reductions in axon diameter, in the size of the cell body, and in the connection of the spinal cord neurons with central terminals of afferents. A few months after nerve damage some neurons begin to die, most of these neurons are C fibers (Sofroniew, Howe, and Mobley 2001).

2.1.5.1.(5). Sympathetic-Somatosensory Reciprocal Stimulation

After stimulation in sympathetic nerve fibers, discharges in afferent fibers in the same nerve root can be observed. It has been thought that sympathetic communication in afferent neurons develops as a result of the increase in alpha-adrenergic receptors in damaged primary axons. The interaction between sympathetic and somatosensory afferents can be observed in neuroma and dorsal root ganglia. In addition, sympathetic axons help depolarization by making basket-like formations around the main cells in the dorsal root ganglia. NGF is thought to be responsible for this event (Castellanos et al. 2003).

2.2. Symptoms and Diagnosis

Neuropathic pain is often expressed by patients with sensations such as electric shock, Burning, Cold, stinging, tickling, itching. Patients are more concerned about abnormal sensations such as electric shock, temperature increase, which they feel in the lethargic area rather than the lethargic area of the painful area. They tend to protect this area from the friction of their clothing, water

contact, and even the breeze of the wind. During physical examination, there are usually no obvious signs, or rarely abnormal neurological signs can be observed. In the area of pain, cold or much rarer redness and heat increase may be felt rarely. Neuropathic pain is a complex formation that has many symptoms and signs, with fluctuations in the number and severity of these symptoms and signs over time (Saito and Yamamoto 1996).

According to IASP, clinicians should make a detailed examination, to diagnose of a patient as NP. Medical history should be taken detailed. Review of systems, and detailed physical and neurological examination should be done.

2.3. Approach to Neuropathic Pain Treatment

2.3.1. Treatment Overview

Although neuropathic pain is a common case in the clinic, the variability in symptoms occurring in similar lesions, difference in response to treatment, and absence of NP syndrome in each patient with nervous system damage or dysfunction are factors that make it difficult to understand NP. Genetic disposition may be the cause of this wide difference. Also differences in the activity of the sympathetic nervous system on peripheral nociceptive fibers and posterior horns, or different conditions in synaptic connections, variable inflammatory response can be the reason (Lineberry and Vierck 1975). These differences make it difficult to diagnose and treat NP. The most frequently recommended treatment modalities in the treatment of NP can be examined under two headings.

- Neurostimulation Therapy
- Pharmacotherapy

2.3.1.1. Neurostimulation Therapy in NP

Neurostimulation therapy is used with increasing frequency in addition to existing medical therapy in many cases. The recommended neurostimulation techniques for pain relief are listed below.

"Transcutaneous electrical nerve stimulation (TENS),

Peripheral nerve stimulation (PNS),
Nerve root stimulation (NRS),
Spinal cord stimulation (SCS),
Deep brain stimulation (DBS),
Epidural motor cortex stimulation (MCS)
Repetitive transcranial magnetic stimulation (rTMS) applications" (Cruccu et al. 2007) .

These techniques differ greatly from each other in terms of intervention, the structures they stimulate, and the bases on which the practices are based upon (Cruccu et al. 2007).

Neurostimulation therapy is being used with increasing frequency in addition to existing drug treatment in many conditions 94. almost free from adverse effects.

TENS is the most used therapy in NP. The principle of the use of TENS at NP is based on the "gate control" theory. In this technique, large afferent fibers are stimulated so that small pain fibers are blocked in the dorsal horn before projecting into the spinal cord (Ro and Chang 2005).

2.3.1.2. Drug therapy (Pharmacotherapy)

Although management of NP is difficult, the most accepted form of treatment is pharmacotherapy. Since NP may not respond partially or completely to common analgesic therapies, adjuvant analgesics (drugs which, have no primary

indication for analgesia) such as antiepileptics, antiarrhythmics, and antidepressants are widely used in the medical treatment of NP (Orza, Boswell, and Rosenberg 2000). Consensus was reached on first-line, second-line, and post-second-line drug therapies, utilizing systematic literature reviews, randomized controlled trial reports, and publications that discuss the evaluation and development of clinical guidelines in order to develop treatment approaches in NP (Vadalouca et al. 2006).

2.3.1.2.(1). First-Line Treatment

2.3.1.2.(1).(a). Anticonvulsants (Gabapentinoids)

The prominent characteristic of NP is neuronal excitability, as in epilepsy. So, antiepileptic drugs are used as first line drug in NP. Many antiepileptic drugs are used effectively in the treatment of NP because they can suppress neuronal hyper excitability by one or more mechanisms (Namaka et al. 2004). They are preferred for diabetic neuropathy and post herpetic neuralgia especially. The gabapentinoids, gabapentin and pregabalin, which is an analogue of gabapentin, lead to decrease the influx of calcium in the terminals of primary afferent neurons by binding to presynaptic voltage-gated Ca^{++} channels in the dorsal horn and reduce the release of excitatory neurotransmitters (such as substance P). Side effects of gabapentin include drowsiness, dizziness, visual blurring, more rarely gastrointestinal symptoms, and moderate peripheral edema (Moulin et al. 2014).

2.3.1.2.(1).(b). Tricyclic antidepressants (Amitriptyline):

Tricyclic antidepressants (TCAs) can be divided into two groups as secondary and tertiary amine compounds. The secondary amine compounds nortriptyline, desipramine and maprotiline relatively selectively block noradrenaline reuptake. The tertiary amine compounds amitriptyline, imipramine and clomipramine block both serotonin and noradrenaline reuptake (Yücel and Çimen). It has been shown that the antineuralgic properties of TCAs are

independent of their antidepressant properties (Max et al. 1987). The effectiveness of TCAs in NP has been shown in many studies. Thus, TCAs act by both increasing posterior horn inhibition and decreasing peripheral sensitization(Beydoun and Backonja 2003). The most common adverse effects of TCAs are dry mouth, anticholinergic effects such as constipation and postural hypotension, sedation and weight gain (Beniczky et al. 2005).

2.3.1.2.(1).(c). Serotonin and norepinephrine reuptake inhibitors (SNRI)

SNRIs inhibit the reuptake of serotonin and norepinephrine in the synapse. Duloxetine inhibits serotonin and norepinephrine reuptake at an equal rate and it should cautiously use in patients with cardiac diseases because it cause increase in blood pressure and cardiac conduction abnormalities. Constipation, nausea, dyspepsia, diarrhea drowsiness, anorexia, and dizziness are the common adverse effects of duloxetine (Kessler 2020).

2.3.1.2.(2). Second-Line Treatment

2.3.1.2.(2).(a). *Tramadol*

Tramadol It is a centrally acting analgesic. It antagonizes mu-opioid receptors, however, its agonistic activity is weaker than morphine and other opioids. Opioid antagonist naloxone inhibits tramadol effects partially. Tramadol is an inhibitor of serotonin and norepinephrine reuptake. Due to its potential for abuse, even though the risk is lower compared with other opioids, tramadol may be limited used (Kessler 2020).

2.3.1.2.(2).(b). Topical or local therapies

Topical treatments have some advantages such as low blood drug levels, fewer side effects and drug interactions comparing to systemic treatments. Topical treatments are preferred in local NPs such as post herpetic neuralgia. This type of preparations is also used in old patients as first-line therapies because of their

differences in drug distribution, metabolism and elimination. Drug level monitoring is unnecessary with local therapies as in systemic use (Kessler 2020).

Lidocaine patches (5%) show their effect by local absorption from the skin and blocking the sodium channels in the periphery. Systemic absorption from the patch occurs in small amounts. There are 3 published studies in which a 5% lidocaine patch was applied in the treatment of NP and achieved successful results. Two of these are related to postherpetic neuralgia (PHN) and the other to focal NP syndromes (Meier et al. 2003).

2.4. Capsaicin

2.4.1. Overview

Capsaicin and capsaicinoids are alkaloid-structured compounds found in the Capsicum family. Capsaicin is the most ingredients responsible for the hot pungent taste of chili peppers. It causes a burning sensation in the mammalian tissue when it comes into contact with (Hayman and Kam 2008). Capsaicin was isolated in 1846 and its structure determined in 1919 (Sawynok 2005). However, it has received increased scientific attention in recent years based on its potential analgesic activity.

2.4.2. Chemistry

Chili peppers contain capsaicinoids which are primarily include capsaicin, dihydrocapsaicin, nordihydrocapsaicin, homodihydrocapsaicin, and homocapsaicin(Fattori et al. 2016). (Figure. 2.1)

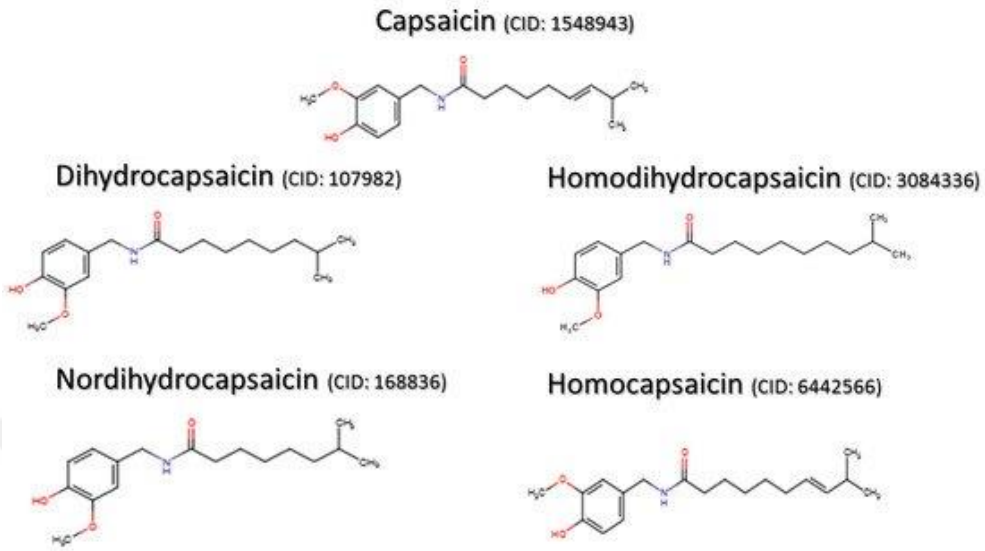

Figure 2.1. "Chemical structure of capsaicin and capsaicinoids" (Fattori et al. 2016).

Approximately 80–90% of the total capsaicinoid content of chili pepper and the most powerful substances among them are capsaicin and dihydrocapsaicin. The content of capsaicinoid of different types of pepper (Capsicum frutescens, annuum and Chinese) has been stated to be 0.22-20 mg / g (Thomas, Schreiber, and Weisskopf 1998).

The chemical name of capsaicin is "trans-8-methyl-N-vanillyl-6-nonenamide" with the molecular formula "C18H27NO3", and molecular weight is 305.40 kDa. It is in crystalline form, colorless and odorless, soluble in fat (lipophilic) and alcohol alkaloid, with a melting point of 62-65 °C (Reyes-Escogido, Gonzalez-Mondragon, and Vazquez-Tzompantzi 2011).

Using The Scoville scale (it is a taste based pungency scale; used to measure the spiciness of chilli peppers), pure capsaicin rates 16 million SHU, habaner: 350,000 SHU, and bell pepper (capsicum): 0–100 SHU (Hayman and Kam 2008).

2.4.3. Capsaicin Pharmacology

Capsaicin has effects on the respiratory, gastrointestinal, cardiovascular, limbic and, thermoregulatory systems which they differ depending on the dose and application rate (Surh and Lee 1995).

2.4.3.1. Pharmacokinetics

After oral administration, capsaicin can be absorbed inactively (50-90%) from the GI tract in animal studies, with peak blood concentrations reached approximately 59 minutes after administration (O'Neill et al. 2012). While oral pharmacokinetics information was limited in human beings. CAP was discovered in the plasma after about 11 minutes and the peak plasma concentration of 2.47 ± 0.13 ng/ml was attained at 47.1 ± 2.0 minutes and CAP was not detected after 90 minutes (Weerapan Khovidhunkit 2009).

Animal studies indicate that CAP is distributed and metabolized vastly by the liver to dihydrocapsaicin by the mixed-function oxidase system. (Arnold, Stewart, and Sammut 2018). It has been suggested that CAP mostly undergoes renal excretion, as both the unchanged and glucuronide form. A little part of the unchanged component is excreted in feces and urine. In vivo animal studies demonstrate that lower than 10% of the administered dose is found in the feces after 48 hours (O'Neill et al. 2012).

2.4.3.2. Mechanism of Action

The mechanism of action of CAP has been widely studied in recent years, (Cortright and Szallasi 2004). CAP stimulates afferent nociceptive neurons and induces hot or burning sensations by releasing SP from afferent nociceptive neurons. It relieves pain by the depletion of substance P which leads up to the desensitization of little afferent sensory neurons(Hayman and Kam 2008).

Nelson determined in 1919 that the chemical structure of CAP was vanilloid (Szallasi and Blumberg 1999). CAP couples to a specified neural

membrane receptor, “Transient Receptor Potential V1 receptor”, TRPV1 (formerly known as “vanilloid receptor, VR1”). Transient Receptor Potential V1 receptors respond to acidosis, temperature, osmolarity and painful stimuli. CAP, heat, protons, and endogenous lipid compounds, known as endovanilloids, directly or indirectly regulate and stimulate “TRPV1”. “TRPV1” has a main function in thermic nociception and inflammatory hyperalgesia (Hayman and Kam 2008).

“TRPV1” is a ligand-gated non-selective cation channel found in the cell membranes of tiny fibrous peripheral nociceptor neurons and vastly found in other tissues such as the intestine, bladder, brain, and kidney. The receptor binds to a nonspecific membrane cation channel that allows Ca2+ and Na+ ions to pass through it. It also includes a heat-sensitive subunit that mediates the hot sensation induced by CAP. TRPV1 is expressed in both the cellular membranes and the endoplasmic reticulum. TRPV1 endoplasmic receptors are separately activated by endovanilloids and it can adjust intracellular Ca2+ levels and this leads to reversible phosphorylation by kinases and phosphatases. When CAP binds to the TRPV1 receptor at little fiber sensory afferent nerve endings, it activates the TRPV1 receptor and triggers an influx of calcium and then releases of inflammatory neuropeptides. After activation of the receptors, neurons are functionally desensitized to more painful stimuli, resulting in analgesia. There could be a degeneration of nociceptive fibers, additionally. The lipoxygenase products of unsaturated N-acyldopamines, anandamidine and arachidonic acid (a cannabinoid) are structurally similar to CAP and have been agonistic activity to TRPV1 receptor endogenously. They activate TRPV1 receptors, so they called as endovanilloids. These endogeneous substances desensitizate of receptors induced by CAP in infected tissue, leading to prolongation of the CAP analgesia in “inflammatory pain” situations. It has been reported that TRPV1 could also be activated and sensitized by extracellular cations (e.g. Na^{+}, Ca^{+2}, and $Mg^{+2)}$. These cations with physiological concentrations participate to “bradykinin-intermediate TRPV1 activation” and heat sensitization of the receptor. CAP controls these

voltage-activated calcium channels by dephosphorylation. CAP reduces inflammatory hyperalgesia by this mechanism. Hagenacker investigated that CAP differently modulates voltage-activating calcium channels in the dorsal root ganglia in mice. In the dorsal root ganglia, small fibrous neurons were more sensitive than large fibrous neurons. These effects may be thought as an added mechanism for vanilloids modified pain signaling at the spinal cord level (Hayman and Kam 2008).

2.4.3.3. Adverse effects

Despite uncertain reports, oral CAP has various adverse effects, especially on the stomach and intestines (gastrointestinal system). The symptoms include cramping, diarrhea, nausea and heartburn (Hammer and Vogelsang 2007). Topical CAP causes irritation and burning and this is the main limitation of using CAP as a topical treatment. There are some incompatible results related the effect of CAP on the gastric mucosa. In some studies it has been showed a gastric bleeding, while others have showed gastro protective properties (Mózsik, Szolcsányi, and Rácz 2005). Additionally, Some have discovered CAP as to prevent gastric cancer and others have discovered a relationship between gastric cancer and ingestion of CAP (Bode and Dong 2011).

2.4.3.4. Toxicity

Oral toxicity of CAP is considered 118.8 mg/kg for male mice and 97.4 mg/kg for female mice. The main toxic symptoms in mice were staggering gait, salivation, stomach damage, skin erythema, cyanosis and bradypnea. Tremors, shortness of breath (dyspnea), a lateral or prone position clonic convulsions, and subsequently died 4 to 26 minutes after dosing were observed in some animals. The reason of death due to CAP may be hypotension and respiratory paralysis (Saito and Yamamoto 1996).

2.4.3.5. Pharmacological actions of capsaicin

2.4.3.5.(1). Pain relief

There are preparations of CAP obtained from hot red pepper that can be applied to the skin in the form of cream. It is known that CAP is effective in the transmission of pain stimuli in the central nervous system and by reducing the substance P, that plays a role in inflammation. Substance P is a neurotransmitter substance released from sensory C-fibers. The antagonist of substance P is CAP. Thus, CAP extracts are used locally to relieve pain in shingles causing nerve injuries and extreme pain. Topical use 4 times a day caused an increase in local temperature and analgesic effect have started within 1-2 weeks. It can also be used to treat conditions such as osteoarthritis, rheumatoid arthritis, fibromyalgia, diabetic neuropathy and chronic nonspecific pain. The repeated use of CAP causes the P substance to be released less like a resistance mechanism. It has been reported that substance P caused a specific blockage in its synthesis and transport, and it relieves pain for a long time by increasing the pain threshold. Desensitization effect can be explained as a reversible effect. Pharmacodynamics studies have reported that topical capsicum preparations are very useful to treat musculoskeletal diseases and NP with or without inflammatory components (Mason et al. 2004).

2.4.3.5.(2). Weigh reduction

CAP increases metabolism by stimulating the sympathoadrenal system. CAP ingestion reduces body fat mass in a dose-dependent method. CAP is studied for obesity treatment (Yoshioka et al. 2004).

2.4.3.5.(3). Diabetes

CAP does not only have effects on body metabolism but also may have advantageous effects on glucose and insulin homeostasis thus diabetes. Dietary and CAP supplementation have an effect on glucose and insulin levels in human being. Regular consuming of capsaicin-containing peppers reduces postprandial

hyperinsulinemia in adults who have a good health (Yuan et al. 2016). Oral administration of 0.015% CAP supplementation to hereditarily overweight mice fed a high-fat regimen lowered glucose and triglyceride levels in plasma during fasting(Kang et al. 2011). Dietary CAP has been shown to reduce obesity caused by glucose intolerance by repressing inflammatory responses and promoting oxidation of fatty acid in adipose tissue and/or liver, the two of which are significant peripheral tissues that affect resistance of insulin (Kang et al. 2010).

2.4.3.5.(4). Cardiovascular effects

The cardiovascular system has a lot of capsaicin-sensitive sensory nerves which play a main role in controlling cardiovascular function by releasing substance P and CGRP ,which is one of neurotransmitters that most powerful vasodilators and plays an significant role in regulating blood pressure under both physiological and pathophysiological conditions. CAP stimulates the release of CGRP by activating TRPV1 and therefore reduces blood pressure. Furthermore, Long-term activation of TRPV1 by CAP reduces lipid storage and atherosclerotic lesions in aortic sinus and thoracoabdominal aorta of mice It was reported that regular consuming of chili pepper for about a month increases the serum lipoproteins resistance to oxidation in adults. It was reported that regular consuming of chili pepper for about a month increases the serum lipoproteins resistance to oxidation in adults. This supports that CAP has prevention of coronary heart disease and atherosclerosis (cardiovascular disorders) (Sharma, Vij, and Sharma 2013; Fattori et al. 2016).

2.4.3.5.(5). Capsaicin in Gastric Disorders

Gastrointestinal effects of CAP are dose dependent. CAP stimulates the release of CGRP and activates cyclooxygenase-1 enzymes, COX-1 enzyme has gastro protective effects, by activating TRPV1 at gastric sensory neurons. Every day treatment with 400 μg of CAP, 3 times each day, diminishes ethanol-induced

and indomethacin-induced gastric mucosal harm in humans who have a good health. CAP decreases Helicobacter pylori-induced gastric ulcer by reducing Interleukin-8 product. Epidemiologic investigations of 103 patients with peptic ulcers in China and 190 patients in India show that the consuming of chili pepper is conversely proportional to the occurrence of peptic ulcers, indicating the intestinal protective effects of CAP. In addition to, CAP has been shown to alter brush border membrane permeability, with expanded microvilli length and perimeter, leading to expanded absorptive surface of small digestive tract in rodents. It has also been shown that zinc absorption is increased in rats fed CAP (Fattori et al. 2016; Sharma, Vij, and Sharma 2013).

2.4.3.5.(6). Neurogenic bladder

Urinary tract is rich in TRPV1 receptors (Yiangou et al. 2001). CAP has been used to relieve neurogenic bladder symptoms as an alternative treatment (Foster Jr and Lake 2014). TRPV1 receptors are significant in the pathogenesis of overactive bladder illnesses and its modification can diminish the symptoms. Mice lacking TRPV1 channels had changed urination thresholds showing that TRPV1 channels may assume a part in detecting bladder filling (Daly et al. 2007). Additionally, bladder afferents of CAP-sensitive participate to the reduction of the volume threshold to reflex urination exisit in experiential animals presented to bladder inflammation(Lecci et al. 1998).

2.4.3.6. Summary of available knowledge on disease-related capsaicin activities

CAP has been used in different clinical conditions. CAP was very important to understand TRPV1 channels and physiological and pathological processes.

(Figure. 2.2) summarizes of the current drug actions of CAP activities-related to disease(Fattori et al. 2016).

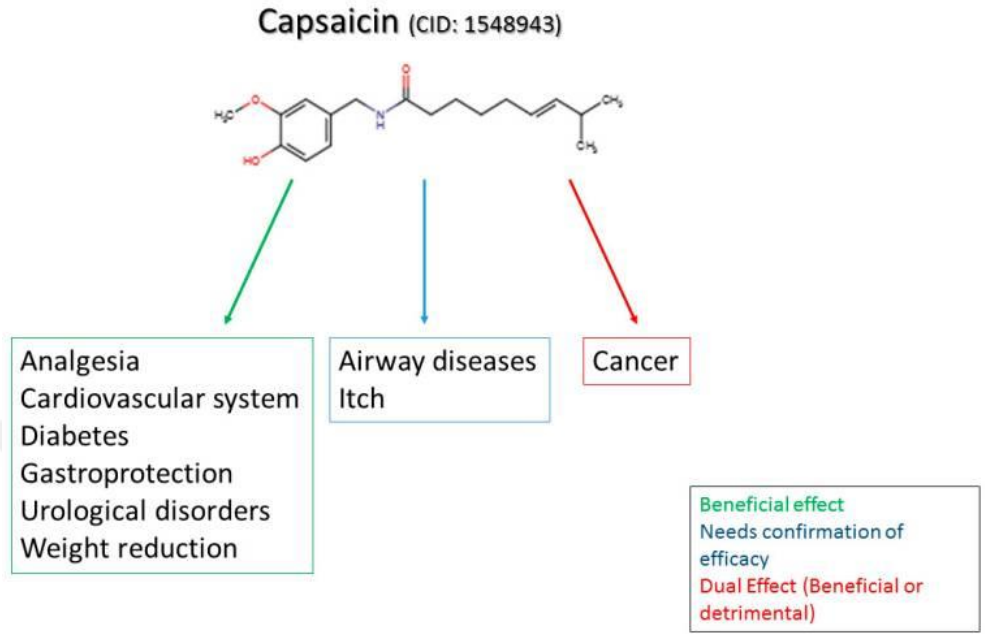

Figure 2.2. The green box shows disorders in which CAP can be used as a treatment and it has beneficial effects. The blue box shows disorders in which the efficacy of CAP remains controversial and the treatment effect of CAP and TRPV1 agonists and antagonists needs more study. The red box shows that CAP can have dual effect, it may prevent or cause cancer (Fattori et al. 2016).

2.5. Drug Delivery Systems (Oral Microemulsions), Bioavailibility

The main disadvantage of CAP is its short half-life and low bioavailability. Another significant one as a medicine is that it gives a burning sensation, which negatively affects patient compliance (Üçeyler and Sommer 2014). Various strategies have been developed to eliminate these disadvantages and ultimately increase its bioavailability. Among these, hydrogel formation, encapsulation in liposomes and iontophoresis gain attention. Recently, nanotechnology has been used to improve sustained-release formulations (Rollyson et al. 2014). These formulations have two advantages: The first one is the sustained and prolonged release to the target organ and the other is the use of lower doses of medicine and thus less adverse effects. Long-acting CAP and capsaicnoids offer promise for the

treatment of chronic pain, atherosclerosis and cancer. The development of drug delivery systems has intensified in recent years to enhance bioavailability of CAP and increase its therapeutic index. Drug delivery systems have generally been developed for transdermal and oral administration of CAP(Sharma, Vij, and Sharma 2013; Peppin and Pappagallo 2014).

The issue of drug solubility emerges as one of the most important challenges in the pharmaceutical field. In recent years, lipid-based systems have been used to deliver drugs with low solubility in oral administration. Lipid-based formulations consisting natural and manufactured lipids have generated academic and commercial regard because it is considered as a potential formulation strategy to improve the oral bioavailability of low water-soluble medicines. Drug absorption can be enhanced and normalized by these formulations and this is especially useful for drugs with a low therapeutic index (Bajaj et al. 2013).

In the past years, microemulsion systems have been extensively studied due to their superior advantages such as easiness of preparation, homogeneity, improved solubility and permeability. Microemulsions (ME) are defined as transparent, thermodynamically stable dispersions of oil, water and surfactant, considerably in combination with a co-surfactant. According to their structure they could be divided into water-in-oil (w/o), oil-in-water (o/w) and bicontinuous microemulsions. The (o/w) microemulsions seem to be promising because it can improve the solubility by dissolving components with poor water solubility into an oil phase (Gursoy and Benita 2004). They can also improve the oral bioavailability of lipophilic pharmaceuticals by minimizing the droplet size from 20 to 200 nm and thus rise the rate of absorption(Salimi et al. 2014).

2.5.1. Self-emulsifying Systems

Self-emulsifying systems (SEDDS) are isotropic and thermodynamically durable systems that can form oil / water ME when mixed with water at low speed, composing of oil, surfactant, co- surfactant / ancillary surfactant and drug

components. Lipids and hydrophobic excipients affect the bioavailability, absorption and distribution of the drug after oral administration by various mechanisms. Consequently, these excipients have very important and beneficial effects in increasing the absorption and improving oral bioavailability of low / non-soluble drugs (Gursoy and Benita 2004; Nazzal et al. 2002).

ME formulations ,with droplet size from 20 to 200 nm (Salimi et al. 2014), were developed to increase the solubility, dissolution rate and bioavailability of CAP (Bajaj et al. 2013).

2.5.2. Excipients Used in Microemulsion

2.5.2.1. Oil

The oil is considered as one of the most significant excipients because it can dissolve the required dose of hydrophobic drug or ease self-emulsification. In addition, it increases the hydrophobic fraction of the drug transported through the lymphatic-enteric system, thereby rising absorption from the gastrointestinal tract relying on the molecular nature of triglycerides. To design the self-emulsifying formulations, long and medium chain triglyceride oils with various degrees of saturation were used. Modified or hydrolyzed vegetable oils are extensively used to formulate ME. They are approved for oral administration as they have the advantage of biocompatibility as well as exhibiting better drug solubility properties. Unmodified edible oils cannot dissolve appreciable amounts of lipophilic drugs in addition to the fact that their self-micro emulsification is difficult, so they are not recommended for use in microemulsion. Other appropriate oil phases are digestible or non- digestible oils and fats such as palm oil, olive oil, corn oil, soya bean oil and animal fat. It has reported that more hydrophobic surfactant could act the role of the lipophobic oil in the formulation(Gursoy and Benita 2004).

2.5.2.2. Surfactant

Several surfactants were used to design the ME. However, the selection is limited as there are very few orally acceptable surfactants. Surfactant selection mostly depends on two factors: hydrophilic-lipophilic balance (HLB) and safety. To achieve a high emulsifying property the emulsifier used in the ME formulation should have a high HLB and a high affinity for water. This ensures instant formation of o/w droplets and/or rapid spreading of the formulation in water (good self-emulsifying performance). Concentration of Surfactant usually ranging from 30% to 60% w/w to form stable ME . The non-ionic surfactants are suggested as the first choice in this formation because they have relatively high HLB. They are also less toxic than ionic surfactants but may cause reversible changes in GI lumen permeability. The commonly used emulsifiers are various solid or liquid ethoxylated polyglycolyzed glycerides and polyoxyethylene 20 oleate. Emulsifiers of natural origin are considered safer than synthetic surfactants, therefore it is preferable to use them(Gursoy and Benita 2004).

Surfactants are amphiphilic and they can dissolve comparatively high quantity of lipophilic drug components. The fat mixtures with higher surfactant and co-surfactant/oil ratios lead to ME formation .

There is a connection between the surfactant concentration and the droplet size used. In certain instances, increasing the surfactant concentration could lead to smaller mean size of the droplet. This is due to the localization of surfactant molecules at the oil-water interface, which leads to the stability of the oil droplets. On the other hand, the mean droplet size could rise with rising concentrations of surfactant. This phenomenon could be attribute to the interfacial disturbance caused by enhanced water penetration into the oil droplets mediated by an increase in concentration of the surfactant leading to the expulsion of the oil droplets in the water.(Gursoy and Benita 2004).

2.5.2.3. Co-surfactant

For effective ME a relatively high surfactant concentrations (usually over 30% w/w) of co-surfactants are needed. Ethanol, propylene glycol (PG), polyethylene glycol (PEG), and some other organic solvents are used orally to dissolve larger amounts of either the hydrophilic surfactant or the drug in a lipid base (Gursoy and Benita 2004).

2.5.3. Phase Diagrams

The phase diagram is important to evaluate the effect of various factors on ME formation as well as to determine the dominant ME areas (Yi et al. 2012). The ME region is characterized by constructing ternary-phase diagrams. The fundamental components of ME formation are the oil phase, the aqueous phase and a surfactant. The co-surfactant could be represented as a single component at a fixed ratio to surfactant and treated as a single pseudo-component. The relative quantities of those three components could be represented in a ternary phase diagram (Sharma et al. 2016).

2.5.4. Conventional Oral Formulation and Microemulsion

Although significant interest in MEs is due to the achievement success of Sandimmune Neoral®, Norvir® and Fortovase®, they are not widely applied because of the absence of general formulation guidance and restricted information about how MEs improve bioavailability (Tang et al. 2013).

The pharmacokinetics of the traditional oral formulation of cyclosporine, Sandimmun®, with of the novel ME formulation, Neoral® were compared. It was observed that the bioavailability of cyclosporine from the novel ME formulation was nearly 6.5-fold greater than that of the traditional formulation. Consequently, the utilization of the novel oral ME formulation of cyclosporine would assist to minimize the problems of cyclosporine malabsorption in many patients with external biliary drainage after liver transplantation. This could also reduce hospital

stay as it would reduce the initial use of the intravenous formulation with its associated adverse effects (Trull et al. 1995).

The O/W microemulsions have been developed and characterized for use as solubilizers and oral delivery systems for Amphotericin B, which is poorly soluble in water and in numerous organic solvents. This poor solubility leads to low bioavailability and decreased membrane permeability, which prevents the development of oral formulations. As a consequence, the solubility of Amphotericin B up to 1000-fold was improved (Silva et al. 2013).

To improve the oral absorption of Fexofenadine, novel microemulsion formulation was designed and compared with Fexofen syrup ,commercial syrup, in vitro and in vivo in rabbits. A pseudoternary phase diagram was used to optimize the microemulsion system where the relative bioavailability of Fexofenadine microemulsion compared to commercial syrup in rabbits was approximately 376.76%. This result indicates that the new W/O microemulsion plays an important role in improving the oral bioavailability of poorly absorbed and low permeability drugs (Gundogdu, Alvarez, and Karasulu 2011).

Minghua S. et.al., investigated that the cosolvents (including ethanol, PEG 400 transcutol P, propylene glycol and glycerol in diverse compositions) could affect the stability of rapamycin formulated as self-microemulsifying drug delivery system in addition to contributing to the enhancement of oral bioavailability, and can also reduce the surfactant content (Sun et al. 2011).

To improve oral bioavailability Xuemei W et.al., prepared curcumin-loaded SMEDDS comprising isopropyl myristate, cremophor RH 40 and ethanol where a 1213% increase in relative bioavailability was observed (Wu, Xu, et al. 2011).

ME formulation of clopidogrel, which has poor water solubility and its oral bioavailability is very low (less than 50%) were designed. it has been shown that the solubility of clopidogrel was significantly enhanced, and hence its oral bioavailability was improved (Patel et al. 2010).

A new phospholipid complex SMEDD has been developed to enhance bioavailability of oral etoposide. The synergistic effect resulting the complex and SEDDS led to enhance bioavailability compared to the SEDDS formulation of normal etoposide and a suspension formulation of the etoposide-phospholipid complex (Wu, Guo, et al. 2011).

In vitro release profiles of classic CAP and CAP-loaded ME in three dissolution media in HCl solution (pH 1.2), in phosphate buffer solution (pH 7.4) and in double distilled water. It was observed that in the first 15 minutes approximately 76% of CAP was released from CAP-loaded ME, while approximately 10% of CAP was released from conventional (classic) CAP within 4 hours in those three dissolution media. Therefore, the ME system has the possibility to improve CAP bioavailability as well as prolong CAP residence time in vivoIn addition, in this formulation there is not considerable irritation on the gastric mucosa as observed in rodents Because one of the advantages of the ME system is that it entrapped most of the active ingredient (here, CAP) that has an irritant effect, preventing CAP from coming into contact with the gastric mucosa immediately (Zhu et al. 2015).

Nanotechnology, such as nanoemulsion which ranged from 50 to 150 nm(Choi et al. 2009), has been used to improve the stability and oral bioavailability of hydrophobic materials (Wang et al. 2007). In addition, the effects of CAP can be extended by controlling CAP release using nanoparticles (Kim et al. 2011). Most of the studies have concentrated on the physical and chemical properties, stability and in vitro study of nanoparticles. Furthermore, the in vivo evaluations of CAP nanoemulsion delivery systems are still lacking (Choi et al. 2013).

Choi, Kim et al., concluded that the dose of CAP-loaded nanoemulsions could be significantly reduced because the prepared nanoemulsions showed approximately 125-fold bioavailability with an extended half-life and decreased distribution compared to classic CAP (Choi et al. 2013).

CAP is mainly used in local preparations. However, local (topical) CAP application causes irritation and a burning sensation (Hayman and Kam 2008), increased sensitivity to noxious stimuli and dermal pain induced by CAP (Bode and Dong 2011). Therefore, we aimed in this study to develop an orally effective delivery system, (O/W) MEs, in order to increase the solubility, in vivo bioavailability and decrease adverse effects of CAP.

3. METHOD AND MATERIALS

3.1. Materials

3.1.1. Chemicals

Capsaicin as an active agent, oleic acid as an oil phase, tween® 80 as a surfactant, propylene glycol and ethanol as co-surfactants and water as an aqueous phase

3.2. Method

3.2.1. Preparation of microemulsion formulation

The substances to be used in the ME formulation were carefully selected. For the preparation of a self-emulsifying system, pseudo-ternary phase diagrams of ME were used. Regions such as ME, nanoemulsion, which vary according to the amount and ratio of oil, water and surfactant to be added to the formulation, were determined on the pseudo-ternary-phase diagrams. Different concentrations of surfactants and cosurfactants were studied with Oleic acid, Tween® 80, Propylene glycol, Ethanol.

Tween® 80 were used as a surfactant (S) while ethanol and propylene glycol were selected as the cosurfactant (CoS) in an S/CoS weight ratios of 2:1, 3:2 and 3:1. For fabrication of pseudo-ternary phase diagram, the weight ratio of oil to mixture of surfactant and cosurfactant at each Smix was varied as 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1, respectively. After being equilibrated, the mixtures were assessed visually and determined as being ME, crude emulsions or emulgel. Technologically suitability were determined in the areas studied, and the formulations selected from these regions were evaluated in terms of droplet size and distribution, zeta potential, physical appearance and microscopic aspects.

3.2.2. Characterization of formulation

Globule size, polydispersity index (PDI), zeta potential, pH were measured in terms of determining the physicochemical properties of ME.

3.2.3. Centrifuge and Heating/cooling cycle test

The optimum formulations were subjected to the thermodynamic stability tests, including the centrifugation test and heating–cooling cycle. Thermodynamic stability was assessed by the procedure that three cycles between 4°C and 40°C with storage at each temperature for 48 h were studied. Also, the formulations were centrifuged at 3500 revolutions per minute (rpm) for 30 min.

3.2.3.1. Globule sizes, Zeta potential and PDI

Globule sizes and zeta potential of MEs were measured using Zetasizer nano ZS (Malvern, UK) at 25°C and PDI was reported. Samples were placed in clear one-use zeta cells and results were recorded.

3.2.3.2. pH Measurement

The pH values of the prepared MEs were measured using a digital pH meter at 25°C. All the readings were determined in triplicates and the averages of the measurements were recorded (Momoh et al. 2020).

3.2.4. Experimental Animal and Housing Conditions

Male mice of 25-30 gram weight, older than 8 weeks, obtained from Çukurova University Health Sciences Experimental Research Center (SABIDAM) were used as experimental animals in the studies. The mice used in the experiments were kept with free access to water and food in groups of ten animals per cage and housed in accordance with the standard laboratory conditions and the ethical rules specified in the SABIDAM guidelines. Before starting the experiments, approval was obtained from the Ç.U. experimental animals' ethics committee.

Surgical interventions on mice were performed under aseptic conditions in the Ç.U. Medical Faculty Pharmacology Department Behavioral Pharmacology Laboratory. These mice were housed in a soundproofed laboratory and maintained in a 12 h light cycle room under controlled temperature (23±1°C) after the intervention.

3.2.5. Surgery to induce NP by partial sciatic nerve ligation (Seltzer's model)

The sciatic nerve partial tight ligation (PSL) model was used to induce NP in mice. Intramuscular (IM) injection of ketamine (80 mg / kg) + xylazine (2.5 mg / kg) was administered for anesthesia before the sciatic nerve ligation procedure. The sciatic nerve was exposed at the thigh level after an incision of approximately 1 cm (1/2 to 1/3 of the posterior part of the sciatic nerve) was applied to the biceps femoris and muscle dissection in order to reach the mouse right leg sciatic nerve under aseptic conditions. After this procedure, the sciatic nerve was removed from the underlying tissues, stretched out of the body with beveled forceps, and knotted with 4.0 non-absorbable, silk thread. Finally the incision was closed with the same type of thread. After injury, animals develop protection behavior and licking of the affected limb, suggesting spontaneous pain. Other behavioral changes are also reported in this model one week after surgery and lasting for approximately six weeks. (Seltzer, Dubner, and Shir 1990). NP will be tested two weeks after sciatic nerve ligation with the cold-plate method and von Frey filaments testing. Animals were tested pre-surgery and 1-2 weeks post-surgery. (Figure 3.1.)

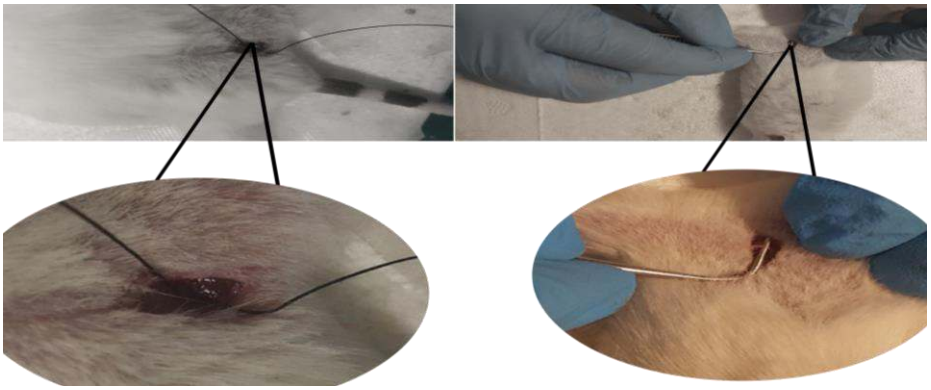

Figure 3.1. Muscle dissection and ligation of the sciatic nerve.

3.2.6. Sham Operation

Under aseptic conditions, after making a 1 cm incision on the biceps femoris muscle and muscle separation, the incision was closed with 4.0 silk thread without seeing the sciatic nerve and knotting it.

3.2.7. NP Behavior

In all groups abnormal movements were seen after one week of surgery. Some mice showed weight loss and a slight reddening of the plantar surface of the toes and some mice also showed associated unpleasant behavior as licking of the affected hind paw, limping with the right paw and enlarged movements(van der Wal et al. 2015).

3.2.8. Experimental Groups and Sequences of Drug Applications:

The mice were divided into six groups according to the experimental protocol. The first group was the group consisting of intact (sound) mice, used as the control group in the graphs, and did not receive any surgery. The second group is called sham, where the sciatic nerve was reached by applying an incision to the skin and muscle tissue, but was closed with silk thread without knotting it. The third group is the neuropathy group. Mononeuropathy was induced in this group of

mice by ligating the sciatic nerve as detailed above. The fourth one was the placebo group in which NP was induced and after 14 days of surgery only placebo was administered. Placebo (oleic acid, Tween 80, propylene glycol, ethanol and water) are excipients without active pharmaceutical ingredients. The fifth one was ME CAP group, the first treatment group, in which NP was induced and after 14 days of surgery 10 mg/kg novel ME CAP were administered by oral gavage. The sixth one was classic CAP, second treatment group, in which NP was induced and 14 days after surgery 10 mg/kg classic CAP were administered by oral gavage (Rollyson et al. 2014; Choi et al. 2013). The ME capsaicin-treated mice group and the corresponding other groups were tested at two hours post oral gavage using Cold Plate (CP) and Von Frey (VF) tests.

3.2.9. Cold Plate Test

Allodynia is one of the most important signs of NP. Thermal stimuli can be used to evaluate allodynia (pain caused by a normally innocuous stimulus)(Allchorne, Broom, and Woolf 2005b). A CP device tests the responses of untethered mice to low temperature stimulation of the plantar side of the paw (Jasmin et al. 1998).

The CP device consists of a circular metal plate that is arranged with a transparent cylindrical plastic glass and it can be fixed at 5 °C. The tested mouse is put on the surface of a metal plate which provides heat or cold stimulation. The degree of nociceptive threshold can be measured via the withdrawal latency when the tested mouse shows sign of lifting or licking hind paws or jumping. (Hot/Cold Plate Analgesia Ugo Basile Italy Model 35150 New Hot/Cold Plate NG, Figure. 3.2).

The automatic time counter, controlled by the pedal attached to the device, determines the time it takes for the experimental animal left on the plate to exhibit one of the specific behaviors. One of the behavioral movements used to determine

this time observationally is the swinging of the mononeuropathy-induced paw or standing up on its hind paws (Ji et al. 2007; Allchorne, Broom, and Woolf 2005a).

In our study, the time, in seconds (sec), until it exhibits any of these behaviors is defined as cold plate latency (CPL). Each mouse was used in an application.

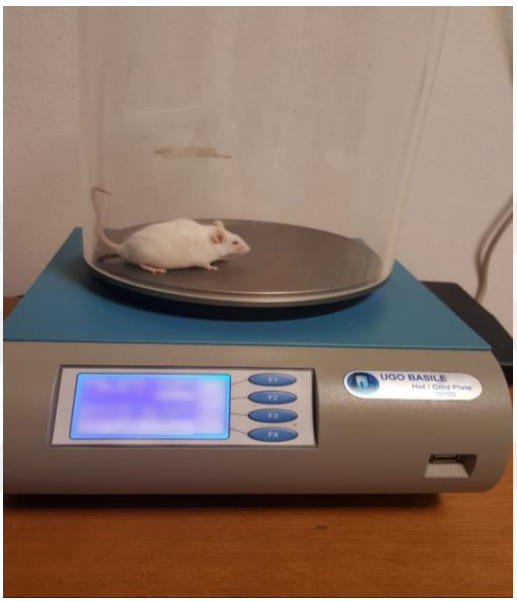

Figure 3.2**.** Cold Plate Device

3.2.9.1. CPL in Animals with Mononeuropathy

After sham and NP operations, the animals were observed in their cages. Their weight and physical appearance were monitored. The neuropathic animals' weights did not differ significantly compared to the control and sham groups. There was also no significant change in their physical appearance, but changes in tissue were observed in the paw on the side of the sciatic nerve ligation (Figure. 3.3.). CPL of the groups were measured 2 weeks after the neuropathy and sham operation. While the CPL of the sham group was not different from the control, a significant decrease was observed in the CPL in the NP group.

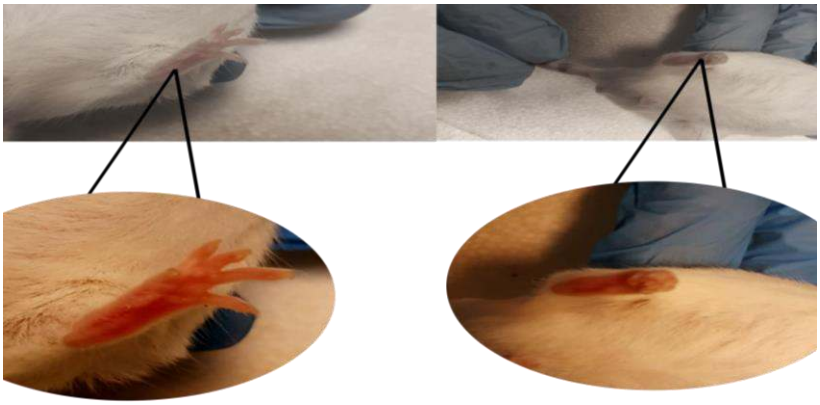

Figure 3.3**.** Physical appearance in paws with and without sciatic nerve ligation

3.2.10. Von Frey Test

3.2.10.1. Equipment

Self-Standing Perforated Metal-Platform, Elevated horizontal wire mesh stand and Plexiglas boxes set on top of the wire grid. The boxes are covered with perforated lids. (Von Frey Hairs, Semmes-Weinstein set of monofilaments, Aesthesio®, Code: 37450-275, Figure. 3.4).

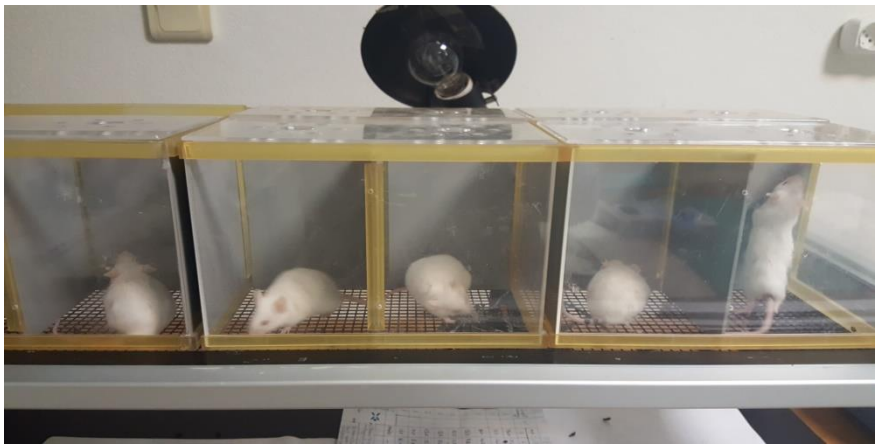

Figure 3.4. Equipment of Von Frey Test

The VF filaments (or hairs) are plastic filaments, with a length of 5 cm and various diameters, fixed on applicators, component of 20 monofilaments, from 0.008 grams to 300 grams. Their end is not sharp but blunt. Rodents exhibit a paw withdrawal reflex when the paw is touched unexpectedly. The Von frey filaments can be used on the Plantar surfaces of a rat or mouse's foot, and the animal will show the sensation by retracting its paw. The VF fibers are applied to the plantar surface of each hind paw with a series of ascending forces. The speed and duration of filament application and the degree of bending can affect the threshold values obtained with this test. With our procedure in mice, the most commonly used filaments are 0.16, 0.4, 0.6, 1, 1.4, 2, 4, 6 and 8 g (Barrot 2012). (Figure. 3.5)

Figure 3.5. Von Frey Hairs

3.2.10.2. Von Frey Testing (Withdrawal Threshold)

The mice are placed in individual transparent boxes with perforations on a raised perforated board made of smooth stainless steel and allowed to acclimate for 15 minutes prior to testing (up to 12 mice can be tested simultaneously). The selected filament is applied to the mid-plantar surface of the left paw (not the lateral borders or bumps that may be more sensitive) (Figure. 3.6). until the

filament is bent. (Figure. 3.7). The procedure is repeated three to five times in succession, then the same procedure is repeated with the right paw. (The expected reaction is a paw withdrawal, sudden flinching, paw pulling back, paw shaking or paw licking). The next animal is tested when we have finished testing the filaments in both paws. If at least 3 expected responses are observed out of 5 trials, the response is considered positive. A specific paw is always tested 3 times. When one or two responses are observed during the 1st three tests, the 4th and 5th tests are performed. The same filament (hair) is applied to the next animals depending on the previous stage. When we have finished testing all the animals, we start over at the 1st animal with the next thicker hair.

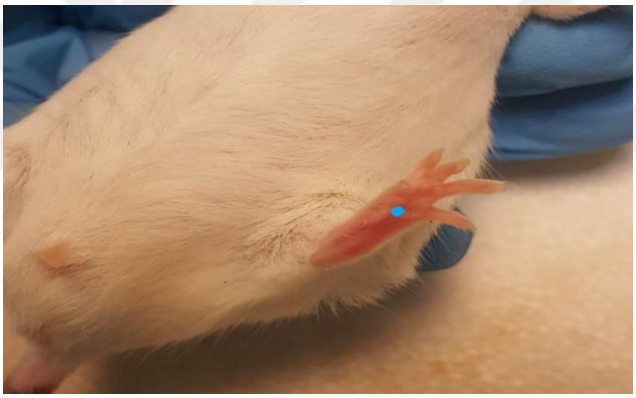

Figure 3.6. Blue dot indicate testing area. Avoid testing the lateral borders

We start the tests with 1 g filament before surgery and with 0.4 g filament after surgery. If a positive response is noticed in the first filament tested, we should test for thinner filament. Each animal is tested until two successive hairs give a positive response. We can say that the paw withdrawal threshold of this animal is the gram value of the lower filament that respond positively (Yalcin et al. 2014).

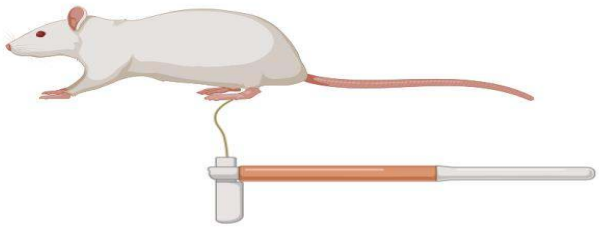

Figure 3.7. Von Frey Testing

3.2.11. Statistical Analysis

Variables were summarized as mean and standard deviation. For comparison of groups, One-way ANOVA was used. Regarding the homogenity of variances, Tukey's test were used for multiple comparisons of groups. All analyses were performed using IBM SPSS Statistics Version 20.0 statistical software package. The statistical level of significance for all tests was considered to be 0.05 (IBM Corp. Released 2011)

4. RESULTS

4.1. Construction of Phase Diagrams

For a good characterization of the ME systems the determination of the pseudo-ternary phase diagram that describes the ideal experimental conditions in which the components must be combined to form a clear preparation is of relevance. The surfactant and co-surfactant were weighed at different ratios (2:1, 3:2 and 3:1) in each tube. The system was stirred using a magnetic stirrer to ensure a thorough mixing at 25°C. Oil and Smix mixtures were titrated, drop-by-drop, with double distilled water while being stirred until the mixture became transparent. The MEs were protected from the by storing in dark-brown bottles wrapped with aluminum foil.

A pseudoternary phase diagram of the investigated quaternary system water/Tween 80/Propylene glycol and Ethanol/ Oleic acid is presented in Table Table 4.1.

Table 4.1. Composition of ME formulations

S/CoS weight ratio	Oil (%)	S and CoS (%)	Water (%)
02:01			
	1,9	35,2	63,0
	5,0	45,0	50,0
	9,4	53,1	37,5
	14,3	57,1	28,6
	19,2	57,7	23,1
	23,1	53,8	23,1
03:01			
	2,5	48,2	49,2
	5,6	50,6	43,8
	10,7	60,7	28,6
	14,3	57,1	28,6
	20,0	60,0	20,0
	24,0	56,0	20,0
03:02			
	2,0	38,0	60,0
	5,1	45,6	49,4
	10,0	56,7	33,3
	13,9	55,7	30,3
	17,4	52,3	30,3
	21,0	49,0	30,1

The phase diagrams have been created by means of a computer program and all trials were replicated three times. The area covered by these points was assumed as ME area in Figure 1. It was monitored that percentage area of ME area in the majority of phase diagrams was largest at S/CoS weight ratio of 2:1 compared to others (Figure. 4.1).

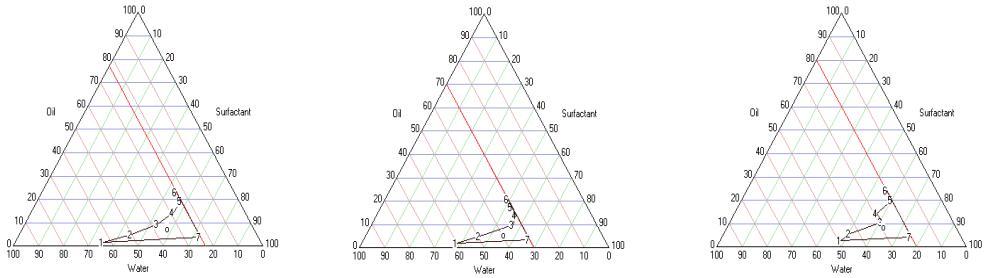

Figure 4.1. Pseudoternary phase diagram of ME formulations

This formulation was used to the next experiments (Table 4.2). Physicochemical characterization was required for appraising the effect of drug loading on ME properties. ME formulations were observed visually for transparency, clarity, phase separation, and undissolved drug. The prepared blank and capsaicin loaded ME was clear, transparent, liquid, single phase, no drug precipitation and with homogeneous appearance.

Table 4.3. Optimum formulation ingredients

Code / Formulation	Oil (%)	Water (%)	S (%)	CoS (%)	Capsaicin(%)
$S_{placebo}$	7.6	34.5	38.6	19.3	-
$S_{capsaicin}$	7.6	34.2	38.6	19.3	3

4.2. Characterization of Formulation

Globule size, PDI, zeta potential and pH were measured in terms of determining the physicochemical properties of ME formulation. Table 3 shows the physicochemical parameters of MEs during the presence and the absence of capsaicin.

Table 4.4. Characterization of ME formulations (mean±SD, n=3)

Code / Formulation	pH	Zeta potential (mV)	Globule size (nm)	PDI
$S_{placebo}$	5.42±0.01	0.56±0.18	124.6±2.8	0.2±0.02
$S_{capsaicin}$	5.31±0.02	0.60±0.36	156.6±3.4	0.3±0.04

The globule sizes and PDI of the prepared MEs were measured by using Dynamic Light Scattering technique. In this technique, a laser light hits the globules in the solution and gets dispersed according to the size of the globules. Globule sizes of capsaicin loaded formulations were slightly larger than the blank formulations, but this difference was not statistically significant ($p > 0.05$).

The pH is probably critical for the stability of the active ingredient but is also critical for the activity of the preservative systems, and any change in pH will likely be indicative of other chemical changes in the formulation. NHS Based on previous studies (Ali and Hussein 2017; Momoh et al. 2020), the pH value of these formulations is suitable for oral administration and there is no significant differences between them. (Table 4.3.)

From the stability point of view, zeta potential, is an important parameter that reflects the stability of the system. The zeta potential refers to the degree of repulsive force between adjacent charged particles in the nanodispersion(Kulshreshtha, Singh, and Wall 2009). Recently, it has been reported that higher zeta potential does not necessarily lead to emulsion stability. However, the rate of decrease in zeta potential actually controls emulsion stability. Increasing the degradation rate of the zeta potential will reduce the emulsion stability. The zeta potential of the sample will determine whether particles in a liquid will tend to flocculate or not. Therefore, knowledge of zeta potential is very useful and effective for emulsion stability study(Bhatt et al. 2010). Also, zeta

potential values of MEs were found almost neutral due to ME components like nonionic surfactants.

4.3. CPL and Von Frey Thresholds (VFTH) in Animals with Mononeuropathy

CPL and VFTH of the groups were measured 2 weeks after the neuropathy and sham operation. Since the CPL and VFTH of the Sham group was not different from the Control, a significant decrease was observed in the CPL and VFTH in the NP group (Figure 4.2, 4.3).

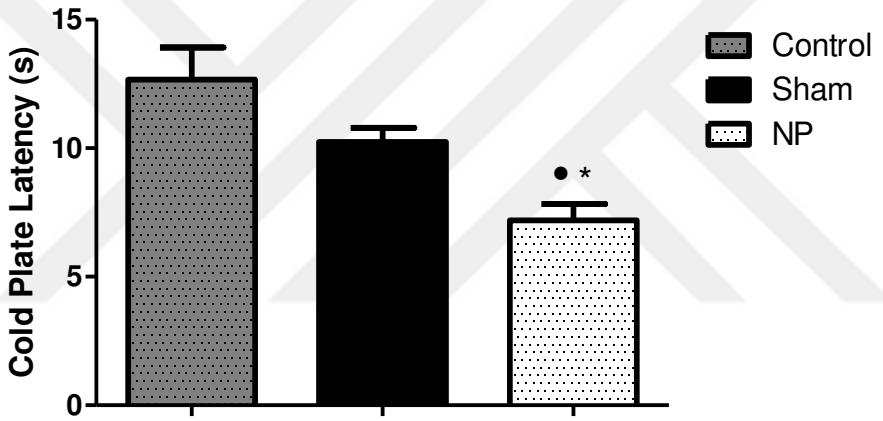

Figure 4.2. CPL latencies in Control, Sham and NP groups. One-way Anova, according to Tukey's Multiple Comparison Test; *: Significantly different from the control group. (P <0.05). •: Significantly different from Sham group. (P <0.05).

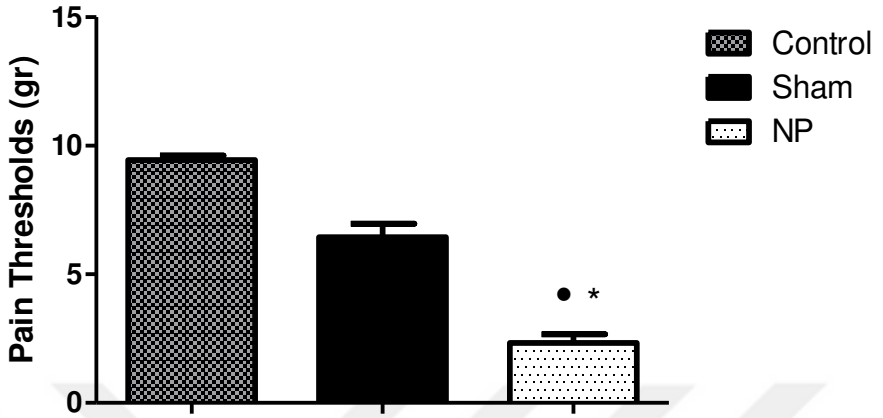

Figure 4.3. VFTH in Control, Sham and NP groups. One-way anova, According to Tukey's Multiple Comparison Test; *: Significantly different from the control group. (P <0.05). •: Significantly different from Sham group. (P <0.05).

4.4. Effects of Novel Microemulsion and Classic Capsaicin Formulation on CP test:

CPL measurements of Novel ME CAP Formulation (CAP, 10 mg for capsaicin or capsaicin+emulsin formulation/kg, oral) and Classic CAP Formulation (Classic CAP, 10 mg/kg, oral) administered to experimental animals with neuropathy (NP) are shown in (Figure 4.4). According to the results obtained that there was no significant difference between CAP and Classic CAP using the Cold Plate test.

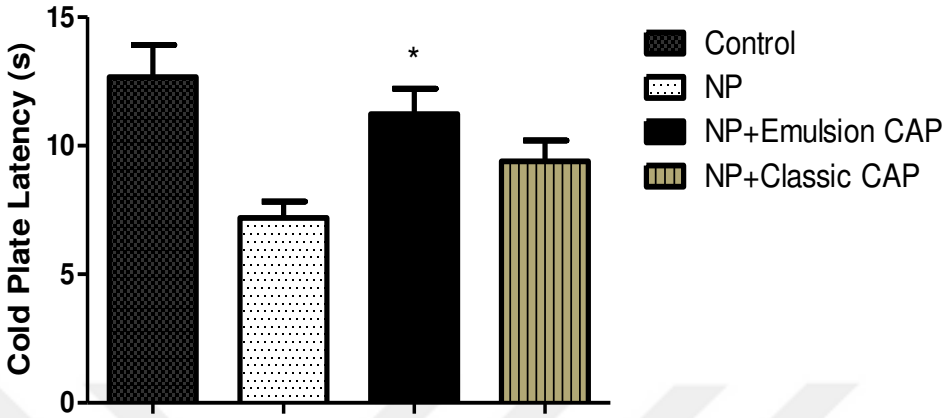

Figure 4.4. The effects of Novel ME and Classic CAP Formulation were compared using the CP test. One-way Anova, According to Tukey's Multiple Comparison Test; *: Significantly different from the NP group. ($P < 0.05$).

4.5. Effects of Novel Microemulsion and Classic Capsaicin Formulation on VF test:

VFTH of Novel ME CAP Formulation (CAP, 10 mg / kg. oral) and Classic CAP Formulation (Classic CAP, 10 mg / kg. oral) administered to experimental animals with neuropathy (NP) are shown in (Figure 4.5). According to the results obtained; a significant increase was observed in the Emulsion CAP Group compared with Classic CAP Group. Thus, Novel ME CAP Formulation is more effective than Classic CAP formulatin using VF test.

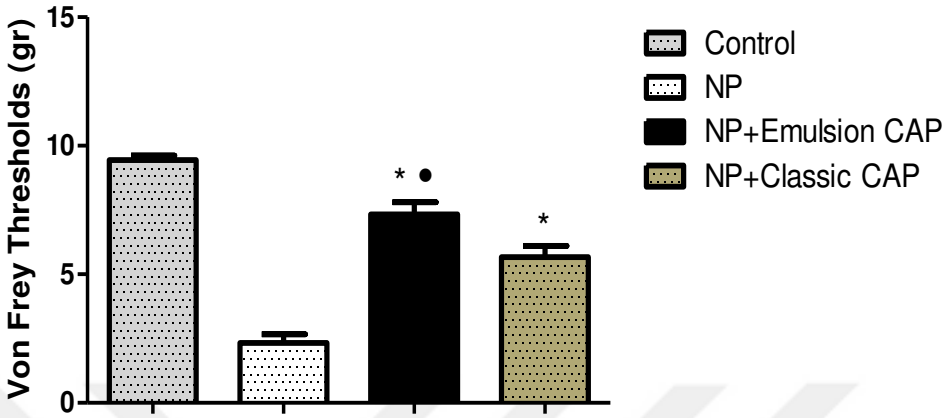

Figure 4.5. *: Significantly different from the NP group. (P <0.05). •: Significantly different from NP+Classic CAP group. (P <0.05).Figure 4.5. The effects of Novel ME and Classic CAP Formulation were compared using the VF test. One-way Anova, according to Tukey's Multiple Comparison Test;

5. DISCUSSION

NP occurs as a result of damage and/or dysfunction of a part of the nervous system. Various animal models have been developed today in order to better understand its pathophysiology and to investigate treatment methods (Dworkin 2002; Kaydok 2009). In our study, the partial tight ligation (PSL) model defined by Seltzer was used to create experimental NP. In this model, mechanical hyperalgesia with prominent and continuous mechanical and cold allodynia developing within the first weeks after the injury continues for six months. Pain behaviors observed in animals in the PSL model are similar to many symptoms in humans with NP syndrome (Seltzer, Dubner, and Shir 1990). In experimental NP models, behavioral symptoms such as allodynia and hyperalgesia have been shown repeatedly (Bennett and Xie 1988). In this study, the effects of capsaicin formulation prepared by classical and ME methods on NP pain induced by sciatic nerve ligation in mice were examined comparatively. This method is used frequently in literature (Seltzer, Dubner, and Shir 1990) and successfully applied by our team in different studies. (Dagilgan, Erdogan, and Aksu 2018; Mete and Aksu 2015). The PSL model is known to be a good animal model for causalgia pains. In our study, the microemulsion form was found to be more effective than the classic form in the VF test. This may be because the PSL model is a good model for touch-sensitive pain.

The CP test which we used in our study is an effective test in determining cold allodynia that occurs in different experimental NP models (Allchorne, Broom, and Woolf 2005a; Ta, Low, and Windebank 2009). Cold allodynia is defined as increased sensitivity to cold stimulus that is not normally painful and is a characteristic feature of clinical NP states (Jørum, Warncke, and Stubhaug 2003). The reason we chose to measure cold allodynia with the CP test to evaluate NP in this study is that it is easy to use. For example, its ability to use a constant cold stimulus, the ability to observe the reaction of the animal to the cold stimulus easily, and the automatic recording of the reaction time.

In our study, it was shown that cold allodynia and mechanical allodynia (Gilron, Baron, and Jensen 2015), which are the most important symptoms of NP, occur as a result of partial ligation of the sciatic nerve. In the CP test, there was no difference between the control and sham groups, but the CPLs of the animals ligated to the sciatic nerve was significantly decreased compared to the control and sham groups. It has been shown that cold allodynia occurs with our method and the results are consistent with the literature and the results of previous studies (Allchorne, Broom, and Woolf 2005b; Ji et al. 2007) Similarly, in the VF test, there was no difference between the control and sham groups, but the CPLs of the animals with sciatic nerve ligated significantly decreased compared to the control and sham groups. It has been shown that mechanical allodynia occurs with our method, and the results are consistent with the literature and the results of our previous studies (Yalcin et al. 2014).

Emulsions are normally thermodynamically unstable system and may separate when exposed to physical tension. Though MEs are visually appear homogeneous as a single-phase system, they are in reality two phase systems. And they were subjected to centrifugation and heating–cooling cycle to support the absence of no separation. Blank and drug loaded formulations did not show any sign of phase separation when subjected to centrifugation, which confirms the physical stability of the ME.

It is well known that globule size of the vehicle is an effective property for drug delivery systems.

MEs, as we mentioned before, seem to be promising because it can increase the solubility by dissolving compounds with poor water solubility into an oil phase. They can also improve the oral bioavailability of lipophilic pharmaceuticals [Dworkin 2002] by reducing the droplet size from 20 to 200 and thus increase the rate of absorption [Benedetti, Vighetti et al. 1998]. However, reduction in globule sizes usually increases the rate of solution. It was observed that the smaller globule size leads to a greater dissolution rate as the proportion of

the surface area exposed to the solvent compared to the volume of the globules increases with decreasing globule diameter.

In the study where we investigated the effects of classical and novel ME formulations of capsaicin on NP pain, we observed that both formulations reduced both cold allodynia and mechanical allodynia. In the cold allodynia test, the novel ME formulation was not superior to the conventional formulation, while in the mechanical allodynia test, the novel ME formulation was found to be more effective than the conventional formulation. In the VF test, the novel ME formulation was found to be statistically significantly more effective than the classical formulation. These results showed that the new oral formulation prepared in this thesis study can be used at a lower dose, and therefore, it may have fewer side effects in neuropatic pains with in touch-evoked allodynia symptoms such as causalgiform pain.

6. CONCLUSION

The results of the study showed that the new oral ME formulation, which was the purpose of this thesis study, is more effective against mechanical allodynia due to NP than the oral form currently used. Based on these results, the effects of the new formulation we have prepared have been found worth examining in more detail with further studies.

The pseudo ternary phase diagram was used to optimize the ME formulations. The present study showed that capsaicin loaded MEs can successfully be prepared with titration method with narrow particle size and PDI range. In conclusion, the developed novel ME formulation improves the solubility of capsaicin, enhances its oral bioavailability and is a promising basis for further development as an oral administration.

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