ANTINOCICEPTIVE ACTIVITIES OF NEW PYRAZOLINE DERIVATIVES IN ACETIC ACID-INDUCED WRITHING MODEL

Master Thesis

Nour Abdullah MUSAOĞLU

Eskişehir 2022

ANTINOCICEPTIVE ACTIVITIES OF NEW PYRAZOLINE DERIVATIVES IN ACETIC ACID-INDUCED WRITHING MODEL

Nour Abdullah MUSAOĞLU

MASTER THESIS

Supervisor: Assoc. Prof. Dr. Nurcan BEKTAŞ TÜRKMEN

Eskisehir

Anadolu University

Graduate School of Health Sciences

June 2022

FINAL APPROVAL FOR THESIS

This thesis titled ANTINOCICEPTIVE ACTIVITIES OF NEW PYRAZOLINE DERIVATIVES IN ACETIC ACID-INDUCED WRITHING MODEL" has been prepared and submitted by Nour Abdullah MUSAOĞLU in partial fullfillment of the requirements in "Anadolu University Directive on Graduate Education and Examination" for the Degree of Master of Science/Master of Arts/Doctor of Philosophy (PhD)/Proficiency in Arts in Pharmacology Department has been examined and approved on …../…../……..

Committee Members	**Signature**
Member (Supervisor) :	.....
Member :	.....
Member :	.....
Member :	.....
Member :	.....

.....

Director

Graduate School of .....

ÖZET

YENİ PİRAZOLİN TÜREVLERİNİN ASETİK ASİT İLE İNDÜKLENEN KIVRANMA MODELİNDEKİ ANTİNOSİSEPTİF ETKİNLİKLERİ

Nour Abdullah MUSAOĞLU

Farmakoloji Anabilim Dalı

Anadolu Üniversitesi, Sağlık Bilimleri Enstitüsü, Haziran 2022

Danışman: Doç. Dr. Nurcan BEKTAŞ TÜRKMEN

Ağrı, hastanın üretkenliğini ve yaşam kalitesini sınırlayan bir sağlık sorunudur. Piyasada birçok analjezik olmasına rağmen, güvenlikleri ve yan etkileri konusunda ciddi endişeler var. Pirazolinler, antienflamatuar ve analjezik ajanlar olarak önem taşımaktadır. Bu tez çalışmasında, yeni sentezlenen Pirazolin türevi 14 bileşiğin periferik analjezik etkisinin araştırılması amacıyla; potansiyel analjezik ajanların antinosiseptif ve antiinflamatuvar özelliklerinin değerlendirilmesi için kullanılan bir yöntem olan % 0,6 asetik asitle i.p., injeksiyonu ile indüklenen kıvranma testi. Bu amaçla ayrı ayrı her bir pirazolin türevinin (AE1-AE12) uygulaması 0.1 ml içinde 10 mg/kg, i.p., dozda olacak şekilde yapıldı. Tüm gruplara i.p. injeksiyon sonrası 30.dk da asetik asit injeksiyonu yapılarak kıvranma testi uygulamasına geçildi. Etkili bir non-steroidal anti inflamatuvar ilaç olan diklofenak pozitif kontrol olarak kullanmıştır. Kıvranma sayısındaki azalma analjezik aktivite olarak değerlendirildi. pirazolin türevi olan AE kodlu 14 bileşikten AE-2, AE-3, AE-4, AE-7, AE-8, AE-9, AE-10, AE-11, AE-12 ve AE-14' ün asetik asit ile oluşturulan ağrı (kıvranma davranışı) davranışını farelerde 10 mg/kg dozlarda anlamlı derecede azaltarak analjezik etki sağlamada başarılı oldukları görüldü. Çalışma bulguları bu Pirazolin türevlerinin abdominal kasılmalarda inhibisyon değerleri diklofenak değerlerine yakın olduğunu göstermektedir. Sonuç olarak, bu sentezlenen Pirazolin türevlerinin analjezik ajan olarak umut vaad edici bir potansiyele sahip olduğunu göstermektedir.

Anahtar Sözcükler: Pirazolin türevi, asetik asit, kavranma testi, ağrı.

ABSTRACT

ANTINOCICEPTIVE ACTIVITIES OF NEW PYRAZOLINE DERIVATIVES IN ACETIC ACID-INDUCED WRITHING MODEL

Nour Abdullah MUSAOĞLU

Department of Pharmacology

Anadolu University, Graduate School of Health Sciences, June 2022

Supervisor: Assoc. Prof. Dr. Nurcan BEKTAŞ TÜRKMEN

Pain is a health problem that limits the patient's productivity and quality of life. Although there are many analgesics on the market, there are serious concerns about their safety and side effects. Pyrazolines are important as anti-inflammatory and analgesic agents. In this thesis study, in order to investigate the peripheral analgesic effect of 14 newly synthesized Pyrazoline derivative compounds; writhing test induced by i.p. injection with 0.6% acetic acid, a method used to evaluate the antinociceptive and anti-inflammatory properties of potential analgesic agents. For this purpose, each pyrazoline derivative (AE1-AE12) was administered separately at a dose of 10 mg/kg, i.p., in 0.1 ml. After the i.p. injection of all groups, acetic acid was injected at 30 minutes and the writhing test was applied. Diclofenac, an effective non-steroidal anti-inflammatory drug, was used as a positive control. The reduction in the number of writhing was evaluated as analgesic activity. It was observed that AE-2, AE-3, AE-4, AE-7, AE-8, AE-9, AE-10, AE-11, AE-12, and AE-14 of 14 compounds with AE code which are pyrazoline derivatives were successful in providing analgesic effect by significantly reducing the pain (writhing behavior) behavior induced by acetic acid in mice at 10 mg/kg doses. The study findings show that the inhibition values of these pyrazoline derivatives in abdominal contractions are close to those of diclofenac. In conclusion, this shows that the synthesized Pyrazoline derivatives have promising potential as analgesic agents.

Keywords: Pyrazoline derivative, acetic acid, writhing test, pain.

ACKNOWLEDGEMENTS

First of all, I would like to extend my heartfelt thanks to my supervisor, Doç. Dr. Nurcan BEKTAŞ TÜRKMEN, who believed in me and granted me the opportunity to complete my master's thesis under her supervision. I thank this unique treasure of knowledge for her immense support and assistance. My thanks also extend to Prof. Dr. Rana ARSLAN, who has been fundamental to this research.

My sincere thanks to the colleagues in the lab, Ilham, Iman, and Dr. Feyza ALYU for their help in completing the laboratory experiments, especially my colleague Ahmed HASAN, who spared no effort in supporting me throughout. Finally, I am grateful for my own efforts, and my children's and husband's patience. They too have endured the uphill struggle alongside me. I dedicate this achievement to my beloved father and my inspiration, my beloved mother, who has not left my thoughts throughout this research, and to my brothers, sisters and friends who have continually motivated me to excel and achieve.

…./…./2022

STATEMENT OF COMPLIANCE WITH ETHICAL PRINCIPLES AND RULES

Bu tezin bana ait, özgün bir çalışma olduğunu; çalışmamın hazırlık, veri toplama, analiz ve bilgilerin sunumu olmak üzere tüm aşamalarında bilimsel etik ilke ve kurallara uygun davrandığımı; bu çalışma kapsamında elde edilen tüm veri ve bilgiler için kaynak gösterdiğimi ve bu kaynaklara kaynakçada yer verdiğimi; bu çalışmanın Anadolu Üniversitesi tarafından kullanılan "bilimsel intihal tespit programı"yla tarandığını ve hiçbir şekilde "intihal içermediğini" beyan ederim. Herhangi bir zamanda, çalışmamla ilgili yaptığım bu beyana aykırı bir durumun saptanması durumunda, ortaya çıkacak tüm ahlaki ve hukuki sonuçları kabul ettiğimi bildiririm.

……..

Nour Abdullah MUSAOĞLU

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS AND ABBREVIATIONS

δ	: Gamma
ATP	: Adenosine Triphosphate
ASICs	: Acid-Sensing Ion Channels
ANOVA	: Analysis Of Variance
ADRs	: Adverse Drug Reactions
CNS	: Central Nervous System
CB1	: Cannabinoid
DMSO	: Dimethylsulfoxide
DDIs	: Drug–Drug Interactions
DH	: Dorsal Horn
GABA	: Gamma-Aminobutyric Acid
i.p.	: Intraperitoneal
IASP	: The International Association for the Study of Pain
MAO	: Monoamine Oxidase
NSAIDs	: Non-Steroidal Anti-Inflammatory Drugs
PNS	: Peripheral Nervous System
PG	: Prostaglandin
PGE2	: Prostaglandin E2
P2X	: Purinergic Receptors
TENS	: Transcutaneous Electrical Nerve Stimulation
TRP	: Transient Receptor Potential
VPL	: Ventral Posterolateral

1. INTRODUCTION AND THE PURPOSE OF RESEARCH

Pain was defined in 1996 by the International Association for the Study of Pain (IASP) as an uncomfortable emotional and sensory experience that can be linked to prospective or actual tissue damage. Pain is a protective mechanism for the body, it acts by preventing tissue damage. It may progress to chronic pain if it persists and becomes maladaptive over time. This type of pain has no protective function and is referred to as pathological pain rather than physiological pain; it is no longer a sign of another disease but rather a separate disorder. Pathological pain is now known as dysfunctional pain. Pain is described as an "experience" rather than nociception. Nociception is the neurological process of noxious information being transduced and conveyed to the brain via a pain pathway. Pain is the outcome of a complicated interaction between higher-order signaling systems and the unique experience of each individual (Steeds, 2016). There are many different types of pain. Allodynia, pain from the stimulation that does not ordinarily produce discomfort, such as pain from a simple touch with postherpetic neuralgia. Dysesthesia is defined as an unattractive aberrant feeling, uncontrolled or created with paresthesia, is not bothersome in those suffering from diabetic polyneuropathy or vitamin B1 insufficiency. Hyperalgesia is described as an exaggerated and frequently painful reaction to a jolt. Hyperesthesia is characterized as an "increased affectivity to provocation, with the exclusion of distinct senses," such as "increasing cutaneous responsiveness to warm sensation without pain."(Saleem and Naz, 2017). Inflammation is the basis of acute pain. It is known that clinically available drugs such as non-steroidal anti-inflammatory relieve the pain due to their anti-inflammatory effects. However, new drug research and development studies continue because of problems such as side effects, tolerability, efficacy, and safety (Aydın, 2002). Chemicals with the pyrazoline ring are one of the most notable compounds in this area. In this thesis, we aimed to investigate the pharmacological activity of pyrazoline derivative compounds given i.p. at 10 mg/kg dosages in mice suffering from peripheral acute pain caused by acetic acid-induced writhing test. The data collected from the implemented writhing test were compared against, the non-steroidal anti-inflammatory drug, diclofenac.

2. LİTERATURE REVİEW

2.1. Pain

Nowadays, pain is one of the most serious concerns that many people encounter. Pain has monetary, social, ethical, and legal consequences. As it is clarified by the IASP "an inconvenient sensory and emotional experience related to, or associated with, actual or possible tissue injury" (http-4).

Mechanisms that cause acute or chronic pain are aspects of the pathophysiology of pain sensation. According to experts, pain is a typical indicator of impending tissue damage. As a result, pain is used as a defense mechanism. Pathologic pain states can occur as a result of peripheral and central nociceptive process disturbances, as well as psychosocial variables. It's critical to distinguish between abnormal discomfort and potential tissue damage. Chronic pain is defined as discomfort that lasts longer than three to six months and affects any region of the body (Dinakar, 2016). Chronic pain can develop if acute pain is not adequately managed, and the cause of chronic pain is often unknown. Chronic pain can have a psychological influence, resulting in anxiety, depression, or the onset of somatization disorders. Chronic pain management must adopt a patient-centered approach to boost patients' well-being and improve their quality of life (Lengel, 2022).

Acute and chronic pain has substantial clinical, economic, and societal consequences. The most prevalent cause of seeing a doctor is pain. According to the Institute of Medicine, more than a hundred million Americans suffer from chronic pain. Missed productivity costs $61.2 billion every year, with over 50 million days of lost work time. Chronic pain costs between $560 to $635 billion in direct and indirect expenditures each year, far more than any of the other six major diseases, including cardiovascular neoplasms, endocrine, metabolic, gastrointestinal, and pulmonary disorders. It is also the leading cause of disability. Pain-related comorbidities put patients and their families under a lot of stress. Overuse, abuse, dependence, and addiction to opioids, as well as depression and poor social skills, are among them (Dinakar, 2016).

Despite the fact that pharmaceutical pain relief is effective for a wide range of pain-related conditions, many patients supplement their therapy with complementary and alternative medicine. Modern analgesics, such as NSAIDs and opiates, have significant clinical limits, particularly with opiates, such as tolerance, addiction, and adverse effects. As a result, many scientists desire to perform experimental research in order to discover safer and more effective analgesics (Zareba, 2009).

2.2. Classification of Pain

Pain is categorized according to mechanisms, symptoms, and syndromes. Thus, pain has been devided into three main categories on a global scale; nociceptive pain, neuropathic pain, and inflammatory pain (Yam et al., 2018).

2.2.1. Based on the mechanism

2.2.1.1. Nociceptive pain

The reaction of the human body's sensory nerve systems to potentially or really damaging stimuli is known as nociception. With the exception of the nervous system, nociceptor receptors may be found in all organs and tissues. Nociceptors detect stimuli, which are then sent to the spinal cord through pain-transmitting nerve fibers and then to the thalamus, where they are interpreted as pain by the cerebral cortex. Nociceptive pain is divided into two kinds, which are visceral and somatic. Somatic pain is carried by sensory fibers, whereas visceral pain is carried by sympathetic fibers. Somatic pain is localized to the injury site and is caused by pain receptors in the bones, muscles, skin, joints, ligaments, tendons, and connective tissue. It's a life-threatening situation. The pain is caused by the somatic nerves, which are well-localized. Due to the low density of nociceptors in the viscera, internal organ pain is difficult to detect and may be reflected pain (Yağcı et al., 2019).

2.2.1.2. Neuropathic pain

The most important contrast between neuropathic and nociceptive pain is that with neuropathic pain, nociceptive stimulation is persistent. The issue begins with a malfunction, which causes the nerve to become mechanically sensitive, resulting in an ectopic impulse. The large and small fibers exchange information. At the same time, critical functions suffer. Central nervous system (CNS) pain: Central neuropathic pain, also known as thalamic pain, post-stroke pain, post-paraplegia pain, and post-quadriplegia pain, is caused by a lesion in the CNS. These are the most difficult pain issues to manage. Damage to the peripheral nervous system causes chronic pain. Neuropathic pain occurs when a neurological structure or function is altered. Two significant hallmarks of diabetes are postherpetic neuralgia and painful neuropathies (Babcan, 2018).

2.2.1.3. Inflammatory pain

Inflammatory mediators, especially prostaglandins, are produced when tissue is injured, causing arterioles to dilate and the region to become red and heated. Capillary and venole endothelium tighten, enabling fluid and cells to flow into the inflammatory region, resulting in swelling. The same mediators contribute to pain. Nociceptors eventually grow hypersensitive to the signals they acquire, resulting in otitis (pain from non-painful stimuli) and hyperalgesia (excessive pain from painful stimuli) (Answine, 2018).

2.2.1.4. Dysfunctional pain

Dysfunctional pain which is a type of chronic pain, has been associated with fibromyalgia, irritable bowel syndrome, and interstitial cystitis, among other medical problems. It is becoming a concerning problem that has a negative impact of unexplained pain on quality of life, the lack of appropriate treatments, and the high cost of health care. The concept of "dysfunctional pain" provides academics with a potentially novel way of thinking about and dealing with pain. Pain amplification, or higher sensitivity to pain, is a feature of dysfunctional pain (Nagakura, 2015).

2.2.2. Based on the duration

2.2.2.1. Acute pain

Acute pain is usually short-lived and accompanied by anxiety or mental distress. It is usually nociceptive and indicates that physical harm has occurred. There is a strong link between the generating injury and the pain in terms of location, intensity, and duration. Some of the reasons include trauma, infection, tissue damage, and inflammation. The most prevalent kind of acute pain is postoperative pain. Acute pain develops chronic pain characteristics after 3-6 months (Saling, 2019).

2.2.2.2. Chronic pain

It is a complex picture in which psychological factors play a role, which is mostly nociceptive and changes the quality of a person's life after the stimulus has passed, leading people to abnormal behaviors. There are no autonomic responses as in acute pain. An increase in sympathetic tone and an increase in neuroendocrine function are evident (Aydın, 2002). The problem with chronic pain is that the pain interacts closely with the patient's thoughts, behaviors, and lifestyles about health and recovery (Babcan, 2018).

2.3. The Physiology of Pain

Pain is produced when nociceptors, sensory nerve endings, engage with unpleasant or harmful stimuli. The consequential nerve impulse transit from the nociceptor to the spinal cord, where it is quickly diverted to the brain through the spinal cord and brainstem neuron tracts. The brain understands the pain and produces a motor response to try to prevent the activity that is causing the suffering (http-3).

2.3.1. Nociceptors

Nociceptors are sensory endings in tissues and organs that are triggered by painful stimuli and are in charge of the early stage of pain feelings. They have high thresholds and only respond to negative stimuli under normal circumstances. Depending on their location, external nociceptors are present in various densities in the skin. They can be found in muscles, joints, bones, and internal organs (Answine, 2018). The epidermis, muscle, joint capsule, bone, and several critical internal organs all have free nerve

terminals. They're utilized to detect possibly harmful chemical, mechanical, and thermal stimuli that may place people at risk (Yam et al., 2018).

Aδ fibers, large and myelinated, are accountable for sudden acute pain. C fibers, small and unmyelinated, are responsible for slow-onset, persistent, or achy pain. These are the primary afferent nerves that comprise these nociceptor. A-fibers are found in both superficial organs like the epidermis and deep somatic tissues like muscles and joints. Heat or mechanical stimuli excite A-fibers, resulting in a prickling, short-term pain sensation. Thermal, mechanical, and chemical stimuli, on the other hand, stimulate C-fibers, leading to a lack of localization and a dull pain sensation. Excitatory, sensitizing, and inhibitory responses are the three principal roles of receptors in primary afferent neurons. Once these receptors have been activated and the pain threshold has been crossed, the resulting impulses are transported along the afferent fibers to the dorsal horn and medulla. Quiet nociceptors are another type of nociceptor. The viscera include silent nociceptors, which lack any anatomical traits that would allow them to respond to noxious stimuli. Chemical mediators generated during inflammatory processes are the sole way to sensitize them (Yam et al., 2018).

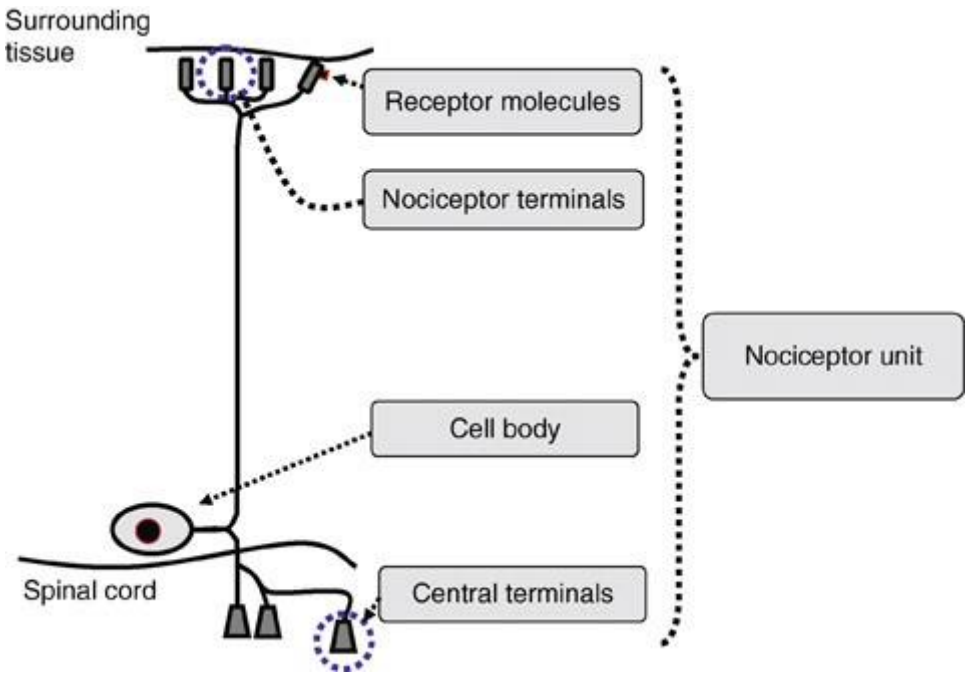


Figure 2.1. *Nociceptor unit (http-9)*

2.3.2. Nociceptive pathways

In the nociceptive pathway, three kinds of neurons are involved:

1. The peripheral nervous system's primary sensory neurons send pain signals from the periphery to the dorsal root of the spinal cord.
2. Spinal cord or brainstem’s secondary sensory neurons that send pain signals to the thalamus.
3. Tertiary sensory neurons, which convey pain signals from the thalamus to the cerebral cortex's somatosensory sections (http- 3).

2.3.3. Pain pathways

Transduction, transmission, modulation, and perception are the four steps in the pain pathways (Dafny, 2020).

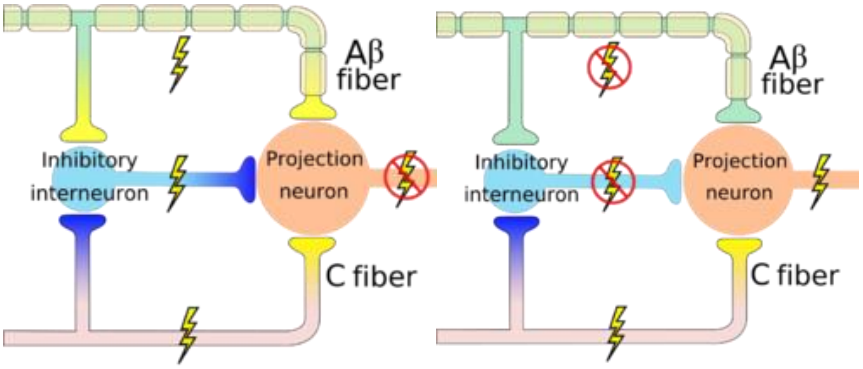
Transduction: The conversion of a tissue physical stimulus into an electrical signal in nerve damage to our tissues, such as a surgical cut or an infectious disease, is transformed into an action potential in a primary afferent neuron during transduction. Prostaglandins are created when tissue injury happens. These inflammatory mediators stimulate or sensitize nociceptors (tissue pain receptors), allowing them to absorb noxious stimuli more quickly. Cells produce potassium (K+), adenosine triphosphate, and hydrogen ions that leads to triggering the nociceptors as soon as tissue injury occurs (Dafny, 2020).

Transmission: The brain receives electrical messages from the nerves. Because voltage-gated Na+ channels open upstream when the threshold potential is reached, the signal is sent up the main afferent axon as an action potential. Dorsal root ganglia is where the cell bodies of primary afferent neurons are found. Main afferent neurons and secondary efferent neurons connect in the spinal cord’s dorsal horn. Within a few levels of stimulation, the action potential produced in the secondary afferent neuron crosses to the other side of the spinal cord and ascends largely inside the spinothalamic tract. The connection between the secondary afferent neurons and the tertiary afferent neurons happens in the thalamus, and tertiary afferent neuron generates action potential that mainly transports to the somatosensory cortex. Together with other cortical regions, this

area regulates emotional, autonomic, and motor responses as well as pain localization (Dafny, 2020).

Modulation: For many years, it was assumed that there is a circuit somewhere in the CNS that may modify incoming pain input. The gate control theory and the ascending/descending pain transmission system are two examples of such a circuit.

Gate control theory: In the mid-1960s, Melzack and Wall proposed the concept of "Gate Control" as the first pain modulation mechanism. Non-painful input closes the gates to painful input, preventing pain sensations from reaching the CNS, according to the gate control theory (i.e., non-noxious input [stimulation] suppresses pain) (Answine, 2019).



Gate closed Gate open

Figure 2.2. *Gate control theory of pain (http-7)*

Ascending and Descending Pain Suppression Mechanism: The Aδ and C fibers (the primary ascending pain fibers) travels from peripheral sites to the spinal cord's dorsal horn to innervate the nociceptor neurons and rise to ascending spinothalamic. At the presynaptic terminals of neurons in the spinal cord, receptors sensitive to opiates are found. Stimulation of these receptors hyperpolarize the neurons, and hypepolirization inhibits the firing and the releasing of substance P. Consequently transmission of pain is blocked (Kanjhan, 1995; Dafny et al., 2020).

Perception: The brain's interpretation of the signal and the production of "pain". When the concerned cortexes inside the brain receive the nociceptive signal, perception happens. The signal is recognized, and an emotional and motor reaction is elicited. It has achieved consciousness and is transitioning from nociception to pain (Answine, 2018).

Central mechanisms of pain perception and modulation: The nociceptive pathways include all the elements of the nervous system whose functions are to detect, transmit, analyze, and control the information generated by a tissue lesion. The nociceptive message starts from the nociceptors, travels along small diameter fibers which are either myelinated (Aδ fibers) or non-myelinated (C fibers), then enters the dorsal horn of the spinal cord which is the first synaptic relay. After crossing from the median plane to the medullary level, the nociceptive fibers will have a second synaptic relay in the ventral posterolateral nucleus(VPL) of the thalamus. VPL neurons exhibit a somatotopic organization and convey action potentials to the somatosensory cortex and associated brain areas (Figure 1).

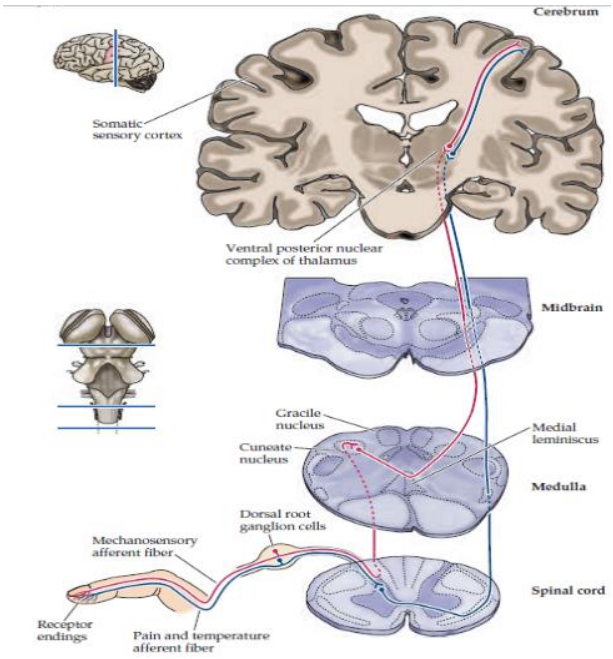


Figure 2.3. *The ascending pathways of the pain (neuroscience, 3rd edition)*

The process of transmission and integration of nociceptive input to the CNS is called "nociception". However, the integration of a nociceptive stimulus at the brain level does not necessarily result in a painful experience. The perception of pain from nociceptive signals occurs in the CNS. It results from the activity of the thalamocortical networks, which process the afferents from the pain pathways (Figure 2).

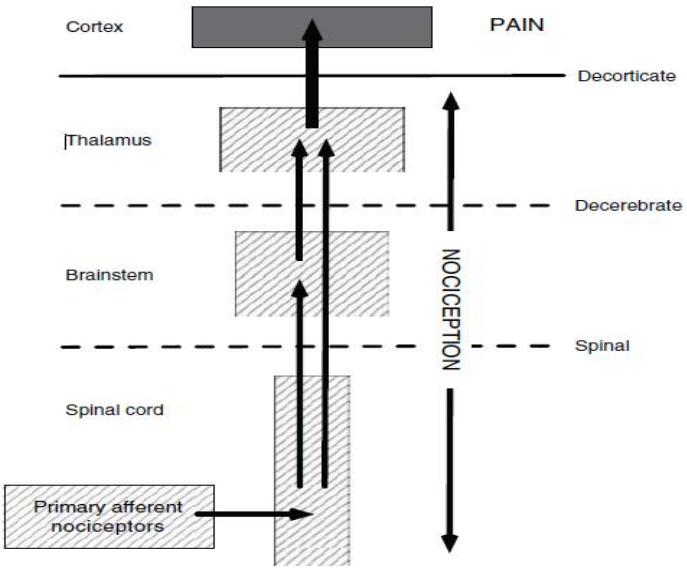


Figure 2.4. *Anatomical distribution of nociception and pain*

Peripheral pain modulation: The first level of pain modulation is acting on the body's peripheral sites. The sensations which originates from the skin, mucous, limbs, and joints are called somatosensation, an it is catogorized as thermoception, nociception, equilibrioception mechanoreception and proprioception. Nociceptors are nerve endings in peripheral nerve cells that sense pain by responding to harmful thermal, mechanical, or chemical stimuli and sending an action potential to the spinal cord and higher levels. When a harmful stimulus happens, the responsible nociceptors act according to the stimulus type, and cyclooxygenase-2 is activated that leads to releasing prostaglandin at the injury's area, and the nociceptors transmit signals to the dorsal horn of the spinal cord (Yam et al., 2018).

2.4. Animal Models of Pain

The multifaceted nature of pain may be investigated by observing intact animals. Animal pain models are created to imitate various clinical conditions in order to better understand the underlying processes and potential remedies. Multiple aspects of pain experience are measured using outcome measures, such as reflexive hyperalgesia, sensory and emotional sides of pain, and the effect of pain on quality of life. The behaviour and the following end-point assessment are both crucial components in nociception animal models. The best models should elicit nocioception by replicating the processes of precise clinical diseases, whether by damage, chemical agents, or other manipulations. Nociceptive behavior's measures must not merely identify pain-like reactions, but at the same time it should do so in a way that is consistent with the pain experience (Gregory et al., 2013).

2.4.1. Reflexive pain tests

Heat, cold, mechanical, and electrical stimuli are utilized to examine evoked behavioral responses in reflexive pain tests. By stimulating nociceptors at the testing location, these reflexive pain tests produce limited, stereotyped motor responses.

Tail Flick test is a technique that evaluates the measurement of the latency to remove the tail from the heat stimulus. Hot-Plate test is another thermal measurment, which tracks the latency for removing or licking the paw from the heated plate. Finally, the Hargreaves test, which includes delivering heat to a rodent's hind paw, was developed to give a more localized evaluation of heat sensitivity. In some pain models, the Hargreaves test has an advantage over the hot-plate test since the control and experimental paw may be evaluated with the same animal.

For asessing allodynia and hyperalgesia behavioral tests of mechanical sensitivity are routinely used. To establish mechanical paw withdrawal thresholds in rodents, von Frey filaments are routinely utilized. Von Frey filament testing is best used to simulate clinical situations with increased cutaneous sensitivity, in neuropathic and inflammatory pains. The evaluation of reflexive pain behavior, in general, has aided the research of underlying processes related to allodynia and hyperalgesia. However, reflexes may not correctly indicate the sensation of pain, yet reflexive pain tests have been accepted for

pain measurement. In recent years, a variety of non-reflexive ways of measuring pain in various illness animal models have been developed (Gregory et al., 2013).

2.4.2. Non-reflexive pain tests

After injection of inflammatory chemicals like formalin, the measurement of paw elevation and licking is one of the most common measures of spontaneous pain behavior. Following the injection of additional drugs like capsaicin and mustard oil, analogous spontaneous behaviors have been quantified. Similarly, writhing behaviors induced by injection of an irritant chemical compound like acetic acid can be used to measure visceral pain models. Chemical stimuli can be adjusted in terms of concentration and volume, and attention to the situation can be tracked (Gregory et al., 2013)

2.4.2.1. Acetic acid-induced writhing test

In the acetic acid-induced abdominal writhing test, irritants are injected into the peritoneal cavity of mice to cause peripheral pain. The animals react by whining or stretching. Writhing can take the form of a stretch, one-sided tension, hind leg extension, belly contraction to the point that the mice's abdomen touches the floor, or trunk rotation (twist). The analgesic effect of the drugs which are being studied is measured by writhing frequency. Acetic acid causes an inflammatory reaction in the abdominal cavity, which stimulates nociceptors. After animals are injected intraperitoneally with acetic acid, the peritoneal region becomes inflamed and unpleasant. Writhing is an outward display of acute pain generated by irritating substances acting on nociceptors, characterized by belly retraction and hindlimb stretching. Irritation causes the production of mediators such as prostaglandins, which increases nociceptors' sensitivity.

The writhing reaction is a reflexive test with no clinical analogues because it can't be done on humans and the emotions involved are unknown. Abdominal contractions induced by acetic acid is a nonselective antinociceptive paradigm because it works by releasing of endogenous mediators that activate neurons that are responsive to NSAIDs, opioids, and other centrally active drugs (Gawade et al., 2012).

2.5. Current Treatment of Pain and New Drug Development

Pain is a complex experience that may be managed with a range of medications. Non-opioid and opioid analgesics are the most prefferded treatment for pain. As a result of the increased focus on pain treatment in recent years, ADRs linked to analgesic use have grown more widespread. Drug-drug interactions can occasionally induce side effects. Analgesic ADRs can be prevented in a majority of cases, resulting in less unpleasant effects for patients. The most commonly used analgesics are non-opioids (paracetamol, metamizole, NSAIDs) and we must be aware of their potential risks. Despite the fact, that we have extensive data and information about them, safety concerns and potential risks have just recently surfaced. Recognizing possible safety hazards is the first step for health professionals in delivering a safe and effective analgesic therapy with minimal risks to patients (Cazacu et al., 2015).

The concept of pain relief as a human right is gaining acceptance on a global scale to help overcome the barriers to appropriate management of pain. One of the most often used and important pain treatments is analgesics. The majority of them are non-opioids and opioids. Antiepileptics, antidepressants, local anesthetics are some other therapeutic classes which might be used in pain therapy (Cazacu et al., 2015).

However, there is a wide range of available therapies in pain management, tremendous progress has been made in medical research in recent years, the management of pain is sometimes inadequate and the treatment of many serious diseases, such as pain and inflammation, is still problematic. Currently used anti-inflammatory and analgesic drugs cause some serious side effects. Therefore, it is crucial to develop potent analgesic and anti-inflammatory drugs with fewer side effects. Today, many drugs that are in clinical use and used for various purposes have a pyrazoline ring in their structure. And this ring is still of interest to researchers.

2.5. Pyrazolines

Pyrazolines are five-membered heterocyclic compounds that include nitrogen. Many techniques have been developed for their synthesis. The discovery of many pyrazoline derivatives with varied biological characteristics has sparked interest in this topic (kaplancikli et al., 2008). It has been claimed that biological assessment of novel bioactive compounds containing the pyrazoline nucleus is critical for the development of potential new analgesics (Sabban et al., 2012).

Many natural compounds, particularly alkaloids, have pyrazolines in their structure. Many antimicrobial, analgesic, anti-inflammatory, anticancer, antidepressant drugs, that are now in clinical use have a pyrazoline ring. Antiepileptic, antitrypanosomal, antiviral, MAO-inhibitory, antinociceptive, insecticidal, hypotensive, nitric oxide synthase inhibitor, antioxidant, steroidal, and anti-diabetic properties are among the other pharmacological effects (sabban et al., 2012). Phenazon (antipyrine) is the first pyrazoline derivative drug active ingredient used in the treatment of algesia and inflammation. Analgesic and anti-inflammatory drugs such as metamizole sodium (dipyrone) and propyphenazone also carry a pyrazoline ring (Alex and Kumar, 2014; Korablina et al., 2016; Nehra et al., 2020). Some pyrazoline-derived drugs, such as dipyrone (metamizole), have been found to have analgesic properties via peripheral mechanisms such as suppression of cyclooxygenase enzyme activity, the arachidonic acid cascade, and prostaglandin production. Other pyrazoline-derived compounds, on the other hand, have been found to behave as centrally acting analgesics. Different pyrazoline compounds with anti-inflammatory or analgesic properties unrelated to opioids, on the other hand, have been identified.

As a result, various molecules having pyrazoline rings have been synthesized in academia and industry, and pyrazoline derivatives with analgesic and anti-inflammatory properties have been discovered (Amir, Kumar ve Khan, 2008, Kaplancikli vd., 2009; Abeed, Jaleel ve Youssef, 2019; Nehra vd., 2020; Mantzanidou, Pontiki ve Hadjipavlou-Litina, 2021).

Pyrazoline Derivatives: Pyrazoline, containing two neighboring nitrogen atoms, is a ring heterocycle. It is basic in nature and has just one endocyclic double bond.

Dihydropyrazoles are another name for them, and their chemistry is similar to that of pyrazoles. The nitrogen atoms of pyrazolines must be numbered 1 and 2 in each structure, according to heterocyclic nomenclature. 1-pyrazoline, 2-pyrazoline, and 3-pyrazoline are the three tautomeric structures for pyrazolines (Figure 2.5). 2-pyrazoline, however, is the most prevalent of these tautomeric complexes.

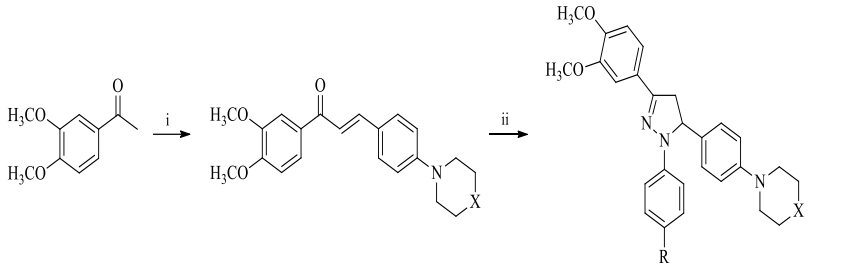
Figure 2.5. *Tautomeric structures for pyrazolines*

Pyrazoline derivatives can provide a wide range of biological activities due to their unique structural characteristics. The most intriguing pyrazoline-type compounds are the 3-substituted pyrazolines. Alkaloids, vitamins, pigments, and components of plant and animal cells are examples of natural heterocyclic compounds.

The pyrazoline ring features a five-membered dihydropyrazole ring structure and an envelope shape, according to X-ray observations.. The carbon atom at position 5 is not in the same plane as the other four atoms in the heterocyclic ring. 2-pyrazoline is water-insoluble but soluble in propylene glycol due to its lipophilic nature. A lot of research has been done on biologically active pyrazolines and their derivatives with various functional groups. Following the pioneering work of Fischer and Knöevenagel in the late 1800s, a classical synthesis of these molecules has been pursued. One of the most frequent techniques to produce 2-pyrazolines is to react,-unsaturated aldehydes and ketones with hydrazines. In this method, hydrazones are produced as intermediates, which may subsequently be cyclized to 2-pyrazolines using an appropriate cyclizing reagent like acetic acid. The discovery of this family of compounds and examination of their potential use as pharmaceuticals is a unique case study in contemporary drug development,

demonstrating the unpredictability of pharmacological activity caused by structural alterations to a prototype medicinal molecule. Pyrazine compounds have a wide range of pharmacological effects and therapeutic applications in general (Sabban et al., 2012).

In this study we aimed to evaluate the periferal antinociceptive effects of 14 novel pyrazoline derivatives which synthesized in Anadolu University Faculty of Pharmacy, Department of Pharmaceutical Chemistry Research Laboratory.



Bileşik	R	X	Bileşik	R	X
1	H	CH_2	**8**	H	O
2	F	CH_2	**9**	F	O
3	Cl	CH_2	**10**	Cl	O
4	CH_3	CH_2	**11**	CH_3	O
5	SO_2CH_3	CH_2	**12**	SO_2CH_3	O
6	SO_2NH_2	CH_2	**13**	SO_2NH_2	O
7	CN	CH_2	**14**	CN	O

Figure 2.6. *Synthesis scheme of pyrazoline derivatives*

(i) 4-(Morpholin-4-yl/piperidin-1-yl)benzaldehyde, 10% NaOH solution, ethanol, room temperature, 10 hours;

(ii) Arylhydrazine hydrochloride, CH3COOH, heating under reflux, 8 hours (Altıntop, 2020)

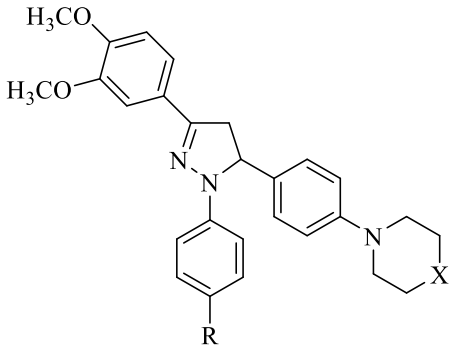


Figure 2.7. *Chemical structure of studed pyrazoline derivatives*

Table 2.1. *AE compounds series*

Bileşik	R	X	Moleküler formülü	Moleküler ağırlığı
1	H	CH_2	$C_{28}H_{31}N_3O_2$	441.58
2	F	CH_2	$C_{28}H_{30}N_3O_2F$	459.57
3	Cl	CH_2	$C_{28}H_{30}N_3O_2Cl$	476.02
4	CH_3	CH_2	$C_{29}H_{33}N_3O_2$	455.60
5	SO_2CH_3	CH_2	$C_{29}H_{33}N_3O_4S$	534.70
6	SO_2NH_2	CH_2	$C_{28}H_{32}N_4O_4S$	520.65
7	CN	CH_2	$C_{29}H_{30}N_4O_2$	466.59
8	H	O	$C_{27}H_{29}N_3O_3$	443.55
9	F	O	$C_{27}H_{28}FN_3O_3$	461.54
10	Cl	O	$C_{27}H_{28}ClN_3O_3$	477.99
11	CH_3	O	$C_{28}H_{31}N_3O_3$	457.57
12	SO_2CH_3	O	$C_{28}H_{31}N_3O_5S$	521.63
13	SO_2NH_2	O	$C_{27}H_{30}N_4O_5S$	522.62
14	CN	O	$C_{28}H_{28}N_4O_3$	468.56

3. MATERIALS AND METHODS

3.1. Materials and Devices Used

Diclofenac (Sigma-Aldrich, St. Louis, MO, USA), acetic acid (Sigma-Aldrich), Dimethylsulfoxide (DMSO) (Sigma-Aldrich). Solvent, ultrasonic bath.

3.2. Experimental Animals

Adult balb-C male mice were used in the experiments. Standard feed pellets and tap water were given to the animals for feeding purposes, housed in well-ventilated rooms at 22 ± 1 ºC with 12-hour light and 12-hour dark cycles. The experimental process was prepared in accordance with the ethical principles of the Declaration of Helsinki and Ethics Committee and approval was obtained by Anadolu University Local Ethics Committee (Decision No: 2018-06).

3.3. Formation of Experimental Groups

16 experimental groups, with 8 mice in each group, were formed by randomly selected mice. The solvent of pyrazolines (AE1-AE12), which is dimethylsulphoxide (DMSO), was used as a test substance for mice in the 1st group and administered at 0.1 mL (*i.p.*). Therefore, the data of this group was evaluated as the control group.

The 2nd group's mice were injected with diclofenac 30 mg/kg in 0.1 mL (i.p.), which was determined as the reference drug (Dikmen et al., 2019), and this group was considered the positive control group. Each pyrazoline derivative (AE1-AE12) was administered separately to the other 14 groups (10 mg/kg V0.1 mL, *i.p)*. The administered doses were determined after a literature review of previous studies about the analgesic effect of pyrazoline (Kaplancikli et al., 2009; Taher et al., 2019). For all groups, acetic acid was injected after 30 minutes of the (i.p.) injection of the pyrazoline derivatives and then the writhing test was performed.

3.4. Acetic Acid-Induced Writhing Test

The writhing test is a chemical method used to induce pain of peripheral origin in mice by injection of irritants such as acetic acid. The analgesic effect of the testing drugs cause a decline in writhing frequency. A writhing movement is characterized by contraction of the abdominal muscles, a backward extension of the hind limbs, and rubbing of the abdomen against the ground (Gawade, 2012). An (i.p.) injection of 0.6% acetic acid solution was applied. At the end of the waiting period of 5 minutes, the above-mentioned abdominal constrictions behavior in each animal were observed and counted for 10 minutes.

3.5. Statistical Analysis

Analysis of data from the writhing test was carried out using one-way analysis of variance (ANOVA) followed by Tukey's HSD multiple comparison test. All statistical analysis results were assessed using GraphPad Prism 5.0 software. The analysis results are stated as mean ± standard error (S.E.M.) and differences between groups were considered significant when $p<0.05$. For plots of graphs, GraphPad Prism 5.0 software was used.

4. RESULTS

4.1. Acetic Acid-Induced Writhing Test

In Figure 4.1. the measured number of writhes in the groups in which AE1-14 coded pyrazoline derivatives were administered at a dose of 10 mg/kg (i.p.) in the acetic acid-induced writhing test is graphically seen. In animals treated with AE-2 (**P<0.01), AE-3 (**P<0.01), AE-4 (*P<0.05), AE-7 (**P<0.01),AE-8 (*P<0.05),AE-9 (*P<0.05),AE-10 (***P<0.001),AE-11(**P<0.01), AE-12 (*P<0.05) and AE -14 (***P<0.001), the number of writhing movements induced by acetic acid was statistically significantly decreased compared to the control group as seen in the group treated with 30 mg/kg diclofenac (***P<0.001).

Table 4.1. As can be seen, 10 mg/kg AE-1-14 and 30 mg/kg diclofenac administration caused a decrease in the number of acetic acid-induced writhing behaviors by 31.82%, 63.64%, 63.64%, 53.16%, 38.18%, 43.16%, 60.91%, 50.44%, 52.25%, 73.64%, 59.09%, 51.35%, 26.8%, 69.55% and 65.91% respectively.

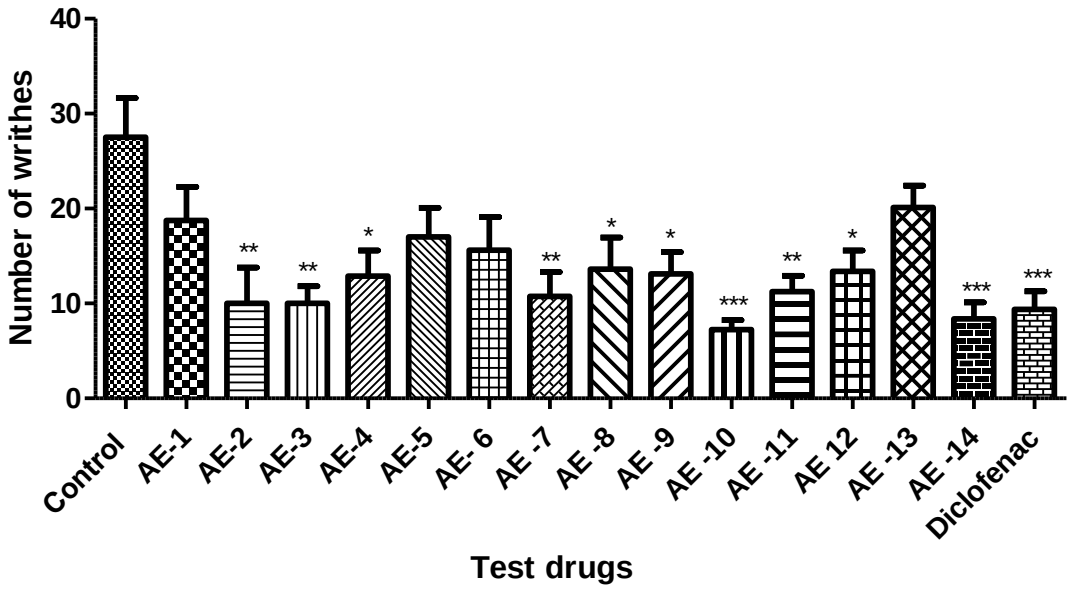


Figure 4.1. *Antinociceptive effect of 10 mg/kg*

*AE-1, AE-2, AE-3, AE-4, AE-5, AE-6, AE-7, AE-8, AE-9, AE-10, AE-11, AE-12, AE-13 and AE -14 and 30 mg/kg diclofenac in acetic acid-induced writhing test. *p<0.05, **p<0.01, ***p<0.001; Significant difference compared to the control group. Values mean ±SE (Standard error). (n=8).*

Table 4.1. *Inhibition % of 10 mg/kg AE-1, AE-2, AE-3, AE-4, AE-5, AE-6, AE-7, AE-8, AE-9, AE-10, AE-11, AE- 12, AE-13 and AE-14 and 30 mg/kg diclofenac on acetic acid-induced writhing test. Inhibition% = [(number of writhes of control group - number of writhes of test group) / number of writhes of control group] x 100 (Jaiswal &Sontakke, 2012).*

Test Drugs	Inhibition %
AE-1	31,81818
AE-2	63,63636
AE-3	63,63636
AE-4	53,16364
AE-5	38,18182
AE-6	43,16364
AE-7	60,90909
AE-8	50,43636
AE-9	52,25455
AE-10	73,63636
AE-11	59,09091
AE-12	51,34545
AE-13	26,8
AE-14	69,54545
Diclofenac	65,90909

5. DISCUSSION

The acetic acid-induced writhing test, a method used to evaluate the antinociceptive and anti-inflammatory properties of possible analgesic agents, was used in this thesis study to examine the peripheral analgesic effect of 14 newly synthesized pyrazoline derivative compounds in mice (Ping et al., 2018). The writhing test is a chemical method that induces peripheral pain in mice by injecting irritants such as acetic acid (Gawade, 2012). The contraction of abdominal muscles, extension of the hind legs, and rubbing of the abdomen against the floor due to contraction characterize the writhing movement (Gawade, 2012). These signs are known as pain signals. The test drug is considered to have an analgesic effect if it reduces these symptoms (Patel et al., 2016). In this thesis study, it is seen that AE-2, AE-3, AE-4, AE-7, AE-8, AE-9, AE-10, AE-11, AE-12, and AE-14 of 14 compounds with AE code, which are pyrazoline derivatives, were successful in providing analgesic effects by significantly reducing the pain behavior (writhing behavior) induced by acetic acid in mice at dose of 10 mg/kg.

Diclofenac, an effective NSAID, is frequently used as a positive control in experimental studies. The pyrazoline derivatives in the thesis experiments showed a similar effect to that of diclofenac which strengthens the data. While diclofenac provided inhibition of pain sensations measured by the number of writhes in abdominal contraction around 65%, the AE-8 coded pyrazoline derivate, which had the least significant effect, provided 50% inhibition. AE-10 and AE-14 surpass the diclofenac effect with 73% and 69% inhibition, respectively. Other statistically significant inhibition values of the pyrazoline derivatives are similar to that of diclofenac. These results indicate that newly synthesized pyrazoline derivatives have potential to be analgesic agents. As a fact, studies carried out with different pyrazoline derivatives support this study. Milano et al. (2008) demonstrated that the pyrazoline derivatives they synthesized modified pain behaviours in formalin and hot plate tests. In another studies using different pyrazoline derivates, successful effects were observed in the acetic acid-induced writhing test (Taher et al., 2019; Mantzanidou et al., 2021).

Although it is seen that different pyrazoline derivatives with similar activity have been synthesized; that the derivatives studied in this thesis catch or even exceed the diclofenac activity level; The fact that patients see and prefer different effects from

different drugs due to reasons such as side effects, tolerability, cost, differences in personal health histories shows that there is always a need for newly synthesized active derivatives. Also, the use of diclofenac-like NSAIDs might cause several issues. NSAID use has been linked to adverse drug reactions such as gastrointestinal bleeding, and cardiovascular and renal effects. ADRs might be caused by drug-drug interactions (DDIs) between the NSAIDs and coexisting medication. For instance, DDIs have been seen when NSAIDs are used with aspirin, alcohol, some antihypertensives, antidepressants, and other widely used medications. In addition, these drugs have different effect profiles in different indications, so they cannot produce effects in every indication. As a result, the remarkable efficacy of the new pyrazoline derivatives studied in this thesis is important.

Though, this study falls short of emphasizing the clinical importance of these derivatives. Following the completion of safety studies, various experimental models must be used for determining the activity, and the mechanism of action should be further investigated and detailed. Because pain management is a complex process involving many chemicals. As a result, it's crucial to test pharmacological effects in different mechanism-based methods, as well as conduct mechanistic studies with agonists/antagonists of the relevant systems. The inhibition of acetylcholinesterase and butyrylcholine esterase enzymes by pyrazoline derivatives used in this thesis study was determined in vitro in a previous study (Altintop, 2016). AE-1 and AE-7 showed the highest level of acetylcholinesterase inhibition in the mentioned study. AE-1 and AE-4 presented similar effects in butyrylcholine esterase inhibition. AE-12 didn’t show any inhibition of acetylcholinesterase, and at the same time AE-8, -9, -10, -11, -12, and -14 didn’t show any inhibition of butyrylcholine esterase. Although these inhibitory activities are effective in relieving pain, they are not major pathways in and of themselves, but they can provide effective analgesia when combined with other pathways. Thus, the antinociceptive effect seen in this thesis study and the previously determined in vitro inhibition activities does not overlap. As a result, by taking into consideration the complex processes which play a role in pain management, it's not surprising that the effect involves multiple pain pathways. Intraperitoneal acetic acid administration to animals in the acetic acid-induced writhing test, which is a sufficient visceral pain model, causes mediators like serotonin, bradykinin, histamine, cytokines, nitric oxide and eicosanoids to be released in the peritoneal fluid and cause an increase in their volume (Mansouri et

al., 2013). Following acetic acid injection, the level of prostaglandins, particularly PGE2, rises in the peritoneal fluid (Ping et al., 2018). The mentioned mediators lower the threshold for nociception and stimulate nociceptive fibers (Mansouri et al., 2013; Coutaux et al., 2005). Because the pyrazoline derivatives used in the thesis study are active in this test model, they may have a peripheral analgesic effect by causing a decrease in the amount of these nociceptive substances or a decrease in the activity of the nociceptors stimulated by these substances. However, it is known that centrally acting analgesics like morphine also reduce the number of writhes and consequently have an effect in writhing experiments. Therefore, it should be taken into consideration for further future studies that pyrazoline derivatives may also employ central mechanisms. As a matter of fact, pyrazoline derivatives have been shown to have neuroprotective effects in various studies, and these effects are mediated by central mechanisms (Ucar et al., 2005; Unsal-Tan et al., 2019). For all of these reasons, as well as in order to determine the pyrazoline effect profile, a detailed mechanism of action analysis should be evaluated in future studies.

6. CONCLUSION AND RECOMMENDATIONS

Based on the literature review, it was determined that compounds containing pyrazoline rings could be effective in pain treatment. Hence, the analgesic activity of the newly synthesized pyrazoline derivatives was demonstrated in an *in vivo* animal model. It is seen that; The antinociceptive activity of each synthesized pyrazoline derivative in the test method used was not similar. While some of them provided diclofenac-like efficacy, some were ineffective. However, in this thesis study, pharmacological activity was carried out using only one animal species and model. In order to fully elucidate the pharmacological effect, an experimental study should be performed on more than one animal species and with more than one experimental method and the effect should be further investigated. In addition, pyrazoline derivatives have been observed to have diverse activities due to the different chemical structures they have. Having different chemical structures produces changes in the pharmacokinetic and pharmacodynamic profiles of pyrazolines. In conclusion, we can say that; the pyrazoline derivatives studied within the scope of this thesis are promising compounds in terms of pain management. By expanding the experimental studies and completing the studies of the mechanism of action, these pyrazolines can be recommended as new drug candidates.

REFRENCES

2001. *Pain*. Reston, VA: National Pharmaceutical Council, Inc.

2004. Purves, D., Augustine, G., Fitzpatrick, D., Hall, W., LaMantia, A., Mooney, R., Platt, M. and White, L., n.d. *Neuroscience*.

2009. *Recognition and alleviation of pain in laboratory animals*. Washington, D.C.: National Academies Press.

Alex, J.M. ve Kumar, R. (2014). 4,5-Dihydro-1H-pyrazole: An indispensable scaffold. J. Enzyme Inhib. Med. Chem., 29 (3), 427-442.

Altıntop M.D. Synthesis, In Vitro and In Silico Evaluation of A Series of Pyrazolines as New Anticholinesterase Agents. *Lett. Drug Des. Discov*. 2020, 17, 574-584.

Amir, M., Kumar, H. and Khan, S., 2008. Synthesis and pharmacological evaluation of pyrazoline derivatives as new anti-inflammatory and analgesic agents. *Bioorganic & Medicinal Chemistry Letters*, 18(3), pp.918-922.

Answine, J. F. A Basic Review of Pain Pathways and Analgesia [Internet]. New York; 2018.

Apkarian, A., Bushnell, M., Treede, R. and Zubieta, J., 2005. Human brain mechanisms of pain perception and regulation in health and disease. *European Journal of Pain*, 9(4), pp.463-463.

Aydede, M., 2017. Defending the IASP Definition of Pain. *The Monist*, 100(4), pp.439-464.

Aydın, N. O. Ağrı ve ağrı mekanizmalarına güncel bakış. Adnan Menderes Üniversitesi Tıp Fakültesi Dergisi, 2002, 3(2), 37-48.

Cazacu, I., Mogosan, C. and Loghin, F., 2015. Safety issues of current analgesics: an update. *Medicine and Pharmacy Reports*, 88(2), pp.128-136.

Colloca, L., Ludman, T., Bouhassira, D., Baron, R., Dickenson, A., Yarnitsky, D., Freeman, R., Truini, A., Attal, N., Finnerup, N., Eccleston, C., Kalso, E., Bennett,

D., Dworkin, R. and Raja, S., 2017. Neuropathic pain. *Nature Reviews Disease Primers*, 3(1).

Coutaux, A., Adam, F., Willer, J. and Le Bars, D., 2005. Hyperalgesia and allodynia: peripheral mechanisms. *Joint Bone Spine*, 72(5), pp.359-371.

Coutaux, A., Willer, J.C., Adam, F., Bars, D. (2005). Hyperalgesia and allodynia: Peripheral mechanisms. Joint Bone Spine. 72 (5), 359–371.

Dafny, N., 2020. Pain Tracts and Sources. *An electronic textbook for the neurosiences*, [online] Available at: <https://nba.uth.tmc.edu/neuroscience/m/s2/chapter07.html> [Accessed 25 May 2022].

Davis, M., 2012. Drug Management of Visceral Pain: Concepts from Basic Research. *Pain Research and Treatment*, 2012, pp.1-18.

Deuis, J., Dvorakova, L. and Vetter, I., 2017. Methods Used to Evaluate Pain Behaviors in Rodents. *Frontiers in Molecular Neuroscience*, 10.

Dinakar, P. and Stillman, A., 2016. Pathogenesis of Pain. *Seminars in Pediatric Neurology*, 23(3), pp.201-208.

Fitridge, R. and Thompson, M., 2012. *Mechanisms of Vascular Disease*. Cambridge: Cambridge University Press.

Fong, A. and Schug, S., 2014. Pathophysiology of Pain. *Plastic and Reconstructive Surgery*, 134, pp.8S-14S.

Gawade, S., 2012. Acetic acid induced painful endogenous infliction in writhing test on mice. *Journal of Pharmacology and Pharmacotherapeutics*, 3(4), p.348.

Gilron, I., 2006. Neuropathic pain: a practical guide for the clinician. *Canadian Medical Association Journal*, 175(3), pp.265-275.

Gregory, N., Harris, A., Robinson, C., Dougherty, P., Fuchs, P. and Sluka, K., 2013. An Overview of Animal Models of Pain: Disease Models and Outcome Measures. *The Journal of Pain*, 14(11), pp.1255-1269.

Kanjhan, R., 1995. OPIOIDS AND PAIN. *Clinical and Experimental Pharmacology and Physiology*, 22(6-7), pp.397-403.

Kaplancikli, Z., Turan-Zitouni, G., Özdemir, A., Devrim Can, Ö. and Chevallet, P., 2009. Synthesis and antinociceptive activities of some pyrazoline derivatives. *European Journal of Medicinal Chemistry*, 44(6), pp.2606-2610.

Kidd, B. and Urban, L., 2001. Mechanisms of inflammatory pain. *British Journal of Anaesthesia*, 87(1), pp.3-11.

Li, J. and Zhang, Y., 2012. Emerging drug targets for pain treatment. *European Journal of Pharmacology*, 681(1-3), pp.1-5.

Mansouri, M.T., Naghizadeh, B., Ghorbanzadeh, B., Farbood, Y. (2013). Central and Peripheral Antinociceptive Effects of Ellagic Acid in Different Animal Models of Pain. *Eur J Pharmacol*. 707(1-3), 46-53.

Mantzanidou, M., Pontiki, E., Hadjipavlou-Litina, D., 2021. Pyrazoles and pyrazolines as anti-inflammatory agents. *Molecules*, 26(11), 3439.

Marcil, J., Walczak, J., Guindon, J., Ngoc, A., Lu, S. and Beaulieu, P., 2006. Antinociceptive effects of tetrodotoxin (TTX) in rodents. *British Journal of Anaesthesia*, 96(6), pp.761-768.

McEntire, D., Kirkpatrick, D., Dueck, N., Kerfeld, M., Smith, T., Nelson, T., Reisbig, M. and Agrawal, D., 2016. Pain transduction: a pharmacologic perspective. *Expert Review of Clinical Pharmacology*, 9(8), pp.1069-1080.

Milano, J., Oliveira, S. M., Rossato, M. F., Sauzem, P. D., Machado, P., Beck, P., ... & Bonacorso, H. G., 2008. Antinociceptive effect of novel trihalomethyl-substituted pyrazoline methyl esters in formalin and hot-plate tests in mice. *European Journal of Pharmacology*, 581(1-2), pp. 86-96.

Nagakura, Y., 2015. Challenges in drug discovery for overcoming 'dysfunctional pain': an emerging category of chronic pain. *Expert Opinion on Drug Discovery*, 10(10), pp.1043-1045.

Nehra, B., Rulhania, S., Jaswal, S., Kumar, B., Singh, G. and Monga, V., 2020. Recent advancements in the development of bioactive pyrazoline derivatives. *European Journal of Medicinal Chemistry*, 205, p.112666.

Nieto, F., Vuckovic, S. and Prostran, M., 2020. Editorial: Mechanisms and New Targets for the Treatment of Chronic Pain. *Frontiers in Pharmacology*, 11.

Ocaña, M., Cendán, C., Cobos, E., Entrena, J. and Baeyens, J., 2004. Potassium channels and pain: present realities and future opportunities. *European Journal of Pharmacology*, 500(1-3), pp.203-219.

Ossipov, M., Dussor, G. and Porreca, F., 2010. Central modulation of pain. *Journal of Clinical Investigation*, 120(11), pp.3779-3787.

Ping, C.P., Mohamad, T.A.S.T., Akhtar, M.N., Perimal, E.K., Akira, A., Ali, D.A.I., Sulaiman, M.R. (2018). Antinociceptive effects of Cardamonin in mice: possible ınvolvement of TRPV1, Glutamate, and Opioid Receptors. Molecules. 23 (9), 2237.

Reddi, D., Curran, N. and Stephens, R., 2013. An introduction to pain pathways and mechanisms. *British Journal of Hospital Medicine*, 74(Sup12), pp.C188-C191.

Saleem, M. and Naz, H., 2017. Analgesics: New Target and Sources. *Pain Relief - From Analgesics to Alternative Therapies*,

Santenna, C., Kumar, S., Balakrishnan, S., Jhaj, R. and Ahmed, S., 2019. <p>A comparative experimental study of analgesic activity of a novel non-steroidal anti-inflammatory molecule – zaltoprofen, and a standard drug – piroxicam, using murine models</p>. *Journal of Experimental Pharmacology*, Volume 11, pp.85-91.

Saygin, M. and Yağcı, Ü., 2019. AĞRI FİZYOPATOLOJİSİ. *SDÜ Tıp Fakültesi Dergisi*,.

Schaible, H., n.d. Peripheral and Central Mechanisms of Pain Generation. *Analgesia*, pp.3-28.

Shaaban, M., Mayhoub, A. and Farag, A., 2012. Recent advances in the therapeutic applications of pyrazolines. *Expert Opinion on Therapeutic Patents*, 22(3), pp.253-291.

Sneddon, L., 2018. Comparative Physiology of Nociception and Pain. *Physiology*, 33(1), pp.63-73.

Steeds, C., 2009. The anatomy and physiology of pain. *Surgery (Oxford)*, 27(12), pp.507-511.

Taher, A., Mostafa Sarg, M., El-Sayed Ali, N. and Hilmy Elnagdi, N., 2019. Design, synthesis, modeling studies and biological screening of novel pyrazole derivatives as potential analgesic and anti-inflammatory agents. *Bioorganic Chemistry*, 89, p.103023.

Tracey, I. and Mantyh, P., 2007. The Cerebral Signature for Pain Perception and Its Modulation. *Neuron*, 55(3), pp.377-391.

Treede, R., Rief, W., Barke, A., Aziz, Q., Bennett, M., Benoliel, R., Cohen, M., Evers, S., Finnerup, N., First, M., Giamberardino, M., Kaasa, S., Kosek, E., Lavand'homme, P., Nicholas, M., Perrot, S., Scholz, J., Schug, S., Smith, B., Svensson, P., Vlaeyen, J. and Wang, S., 2015. A classification of chronic pain for ICD-11. *Pain*, 156(6), pp.1003-1007.

Ucar G, Gokhan N, Yesilada A, Bilgin AA., 2005. 1-N-Substituted thiocarbamoyl 3-phenyl-5-thienyl-2-pyrazolines: A novel cholinesterase and selective monoamine oxidase B inhibitors for the treatment of Parkinson’s and Alzheimer’s diseases. *Neurosci Lett*., 382, pp.327-331.

Unsal-Tan, O., Küçükkılınç, T. T., Ayazgök, B., Balkan, A., and Ozadali-Sari, K., 2019. Synthesis, molecular docking, and biological evaluation of novel 2-pyrazoline derivatives as multifunctional agents for the treatment of Alzheimer's disease. *Med. Chem. Comm.*, 10(6), 1018-1026.

Yam, M., Loh, Y., Tan, C., Khadijah Adam, S., Abdul Manan, N. and Basir, R., 2018. General Pathways of Pain Sensation and the Major Neurotransmitters Involved in Pain Regulation. *International Journal of Molecular Sciences*, 19(8), p.2164.

Zareba, G. (2009). Phytotherapy for pain relief. *Drugs of Today*. 45 (6), 445-467.

http-1: https://www.researchgate.net/publication/322924533_A_Detailed_Review_on_Nociceptive_Models_for_the_Screening_of_Analgesic_Activity_in_Experimental_Animals (Accessed 20 May 2022).

http-2: https://anesthesiaexperts.com/uncategorized (Accessed 20 May 2022).

http-3: https://www.atrainceu.com/content/4-physiology-pain (Accessed 23 May 2022).

http-4: https://www.iasp-pain.org/publications/free-ebooks/classification-of-chronic-pain-second-edition-revised (Accessed 21 May 2022).

http-5: https://www.britannica.com/science/pain (Accessed 20 May 2022).

http-6: http://fizyoaktif.com.tr/?p=1245 (Accessed 20 May 2022).

http-7: https://www.physio-pedia.com/Gate_Control_Theory_of_Pain (Accessed 21 May 2022).

http-8: https://www.webmd.com/pain-management/guide/pain-types-and-classifications (Accessed 20 May 2022).

http-9: https://link.springer.com/referenceworkentry/10.1007/978-3-540-29678-2_4000 (Accessed 24 May 2022)