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# Neurochemical Mechanisms Of Antinociceptive Effect

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## Abstract

Pain is a very common concern for patients and has a significant effect upon their quality of life. Specifically, pain, as defined by the International Association for Pain Research, is an unpleasant feeling and emotional experience associated with potential or existing tissue damage. Pain can be divided into acute and chronic pain depending upon its duration. Chronic pain, which is defined as pain lasting for longer than 3 months after the onset of the initial injury or disease, affects the quality of daily living. Chronic pain is challenging to treat due to the limited efficacy and adverse side effects of therapies. The International Association for the Study of Pain defines pain as "an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage".

**Key words:** antinociceptive effect, afferent systems, nervous system

## Introduction

Pain is a very common concern for patients and has a significant effect upon their quality of life. Specifically, pain, as defined by the International Association for Pain Research, is an unpleasant feeling and emotional experience associated with potential or existing tissue damage. Pain can be divided into acute and chronic pain depending upon its duration. Chronic pain, which is defined as pain lasting for longer than 3 months after the onset of the initial injury or disease, affects the quality of daily living. Chronic pain is challenging to treat due to the limited efficacy and adverse side effects of therapies [1]. The International Association for the Study of Pain defines pain as "an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage". It can result from the direct activation and sensitization of the primary sensory neurons by autacoids such as prostaglandin, proinflammatory cytokines, and chemokines. In this case, it is known as inflammatory pain. It can also arise from central sensitization, independent of the stimulation of the sensory terminals [2]. The rostral ventromedial medulla is the final common output node of the complex, brain-spanning network that affects the experience of pain. Spinal cord-projecting neurons terminate in the dorsal horn where nociceptive processing is affected, resulting in accentuation or attenuation of withdrawal reflexes. These spinal projections also control the processing of ascending transmission, thereby influencing affective and perceptive dimensions of pain.

Depending on the strength of electrical stimulation in the rostral ventromedial medium, nociception is either facilitated or inhibited. Within the rostral ventromedial medulla, serotonergic, γ-aminobutyric acid-mediated [GABAergic], and perhaps also glutamatergic neurons can modulate pain. Pain stimuli are associated with heightened activity of some rostral ventromedial medulla neurons and lowered activity of others [so called ON and OFF cells].

In addition, μ-opioid receptors important for opioid analgesia are present in virtually all neural substrates in the brain contributing to the experience of pain, and consistently there is experimental evidence for the involvement of spinal as well as multiple supraspinal sites in analgesia [3]. Serotonin is critically involved in neuropathic pain. However, its role is far from being understood owing to the number of cellular targets and receptor subtypes involved. In a rat model of neuropathic pain evoked by chronic constriction injury of the sciatic nerve, the role of 5-HT2B receptor in dorsal root ganglia and the sciatic nerve was studied. The 5-HT2B receptor activation both prevents and reduces chronic constriction injury-induced allodynia. Activation of peripheral 5-HT2B receptor both prevents and reduces mechanical allodynia induced by chronic constriction of the sciatic nerve via blood-derived macrophage bearing the receptor [4]. Pain is a noxious sensation resulting from tissue injury and acts as a beneficial response necessary for the preservation of tissue integrity. Clinical treatment of pain currently involves the use of opioid and non-opioid agents. However, both types of drugs present safety profiles with limited effectiveness and numerous side effects. Non-steroidal anti-inflammatory drugs are associated with severe gastrointestinal, renal or liver damage. To date, opioids are the most potent and effective analgesics for pain treatment. However, their severe side effects, such as tolerance and addiction, limit their use. Therefore, the search for new analgesics with higher efficacy and fewer perceived effects is a continuous objective for pain treatment [5].

**Results and discussion:**

During the last few years novel derivatives of 5-HT were described and presented interesting antinociceptive and anti-inflammatory properties. For instance, C18 5-HT (βN-octadecanoyl-5-hydroxytryptamide) was found in the surface wax of green coffee beans and was synthesized by combining an octadecanoyl unit with serotonin. Some amides in the serotonin class have also demonstrated an anti-inflammatory effect by inhibiting the expression of caspases, a class of enzymes involved in the inflammatory process6. Furthermore, Ortar et al. described that another serotonin amide, N-arachidonoyl 5-HT, has agonist activities towards CB1 and CB2 cannabinoid receptor type, as well as being an antagonist of TRPV receptors, confirming its analgesic effect. Intraplantar administration of formalin produces nociception characterized by two distinct phases [5]. The first phase (neurogenic phase) occurs between formalin injection and 5 minutes and is due to activation of C-fibers with activation of TRPA1 channels and reflects centrally mediated pain. The second phase (inflammatory phase) occurs between 15 and 30 minutes after formalin injection and is mediated by the release of a combination of inflammatory mediators and sensitization of central nociceptive neurons. It is also well-known that centrally acting drugs, such as opioids (morphine and codeine), inhibit nociception in both phases, while peripheral-acting drugs, such as indomethacin and acetylsalicylic acid, inhibit only the second phase. However, there are conflicting data in literature relating non-steroidal anti-inflammatory drugs acting in the first phase of the model. It is known that the first phase is also mediated through liberation and/or synthesis of histamine and serotonine. So, such drugs that can direct or indirect act interfering with both pathways can also affect and reduce the first phase of formalin induced linking response. C18 5-HT significantly decreased the duration of licking time at the two higher doses in both phases of pain responses in the formalin-induced licking model [5]. Serotonin activity in the brainstem is primarily under the control of 5-HT1A somatodendritic receptors, although some data also suggest the involvement of 5-HT1B receptors. Paracetamol (acetaminophen) has been extensively studied as analgesic for pain relief in many clinical settings, but its mechanism of action still is under considerable debate. Paracetamol crosses the brain barrier, and many reports indicate that paracetamol exerts its antinociceptive activity not only peripherally, but also within the central nervous system. In addition, paracetamol also exhibits antinociceptive effects in tests that are reputed to be sensitive only to central analgesics, as hot-plate test and tail-flick test, and intracerebroventricular or intrathecal administration of paracetamol have also been shown to provide antinociception [6]. Nevertheless, paracetamol can trigger side effects when taken regularly. Combined therapy is a common way of lowering the dose of a drug and thus of reducing adverse reactions. Since β-caryophyllene oxide (a natural bicyclic sesquiterpene) is known to produce an analgesic effect, this study aimed to determine the anti-nociceptive and gastroprotective activity of administering the combination of paracetamol plus β-caryophyllene oxide to CD1 mice. Anti-nociception was evaluated with the formalin model and gastroprotection with the model of ethanol-induced gastric lesions. According to the isobolographic analysis, the anti-nociceptive interaction of paracetamol and β-caryophyllene oxide was synergistic. Various pain-related pathways were explored for their possible participation in the mechanism of action of the anti-nociceptive effect of β-caryophyllene oxide, finding that NO, opioid receptors, serotonin receptors, and K+ATP channels are not involved. The combined treatment showed gastroprotective activity against ethanol-induced gastric damage. Hence, the synergistic anti-nociceptive effect of combining paracetamol with β-caryophyllene oxide could be advantageous for the management of inflammatory pain, and the gastroprotective activity should help to protect against the adverse effects of chronic use [7]. Also, the analgesic effects of paracetamol are attenuated by drugs that act via inhibition of serotonergic, opioid and cannabinoid systems suggesting that a number of neurotransmitter system may be involved in the central antinociceptive mechanism of paracetamol, in particular, serotonergic pathways. In support of this, different studies have shown that the action of paracetamol is significantly reduced when lesions are produced in the serotonergic pathway or by inhibiting synthesis of serotonin 5-HT in animal models. Conversely, paracetamol treatment induces a significant increase in 5-HT levels in the brainsterm. Another hypothesis that has surfaced is that the analgesic action of systemically administered paracetamol could be attributed to spinal 5-HT (5-HT3 and 5-HT7) receptors mediated the enhanced neurotransmitter release in the descending serotonergic pathway, which is responsible for modulation of pain at the spinal level. However, other studies report a serotonergic facilitatory modulation onto the spinal cord through 5-HT3 in different pain models [6]. The precise mechanism of action of paracetamol is still unknown. It is reported to involve the inhibition of cyclooxygenases (COX-1, COX-2, and COX-3) and an interaction with the endocannabinoid system and serotonergic pathways. Moreover, it acts on transient receptor potential channels and voltage-gated potassium channels, inhibits T-type calcium channels, and affects l-arginine in the NO synthesis

pathway. Paracetamol is metabolized to p-aminophenol and then converted to N-acylphenolamine, the most important mediator of analgesia. It is metabolized to other compounds as well, such as N-acetyl-p-benzoquinone imine, which also appears to produce analgesia [7]. The 5-HT1A receptors have a somatodendritic location on 5-HT neurons of the midbrain raphe nuclei (autoreceptors) and on neurons postsynaptic to 5-HT nerve terminals, mainly in cortico-limbic areas that exerts a pronounced inhibitory influence upon the release of 5-HT throughout the CNS. Also, 5-HT1A can be localised at the spinal cord, a diversity of analgesiometric paradigms has been employed and numerous behavioural studies have reported hyperalgesia upon spinal administration. Stimulation of 5-HT1A receptors also attenuates induction of antinociception by the antidepressant, clomipramine. While some authors have demonstrated that a 5-HT1A agonist, F13640, induced central analgesia in different analgesimetric test [6]. Various pain-related pathways were presently evaluated to explore their relation to the nociceptive effect of β-caryophyllene oxide. One such pathway is related to opioid receptors, located in the central and peripheral nervous system. Their activation inhibits adenylyl cyclase and the production of cAMP. The direct interaction of cAMP with different membrane ion channels can modulate pre- and postsynaptic Ca++ currents and thereby attenuate the excitability of neurons and/or reduce the release of pronociceptive/proinflammatory neuropeptides. In addition, opioid receptor activation leads to the opening of G protein-coupled inwardly rectifying K+ channels, thus preventing neuronal excitation and/or propagation of action potentials. Since the anti-nociceptive effect of β-caryophyllene oxide was not modified by pretreatment with naloxone (a non-selective opioid receptor antagonist), the corresponding mechanism of action in phase II of the formalin test did not involve opioid receptors [7]. Opioids (such as morphine) are the most effective drugs for treating severe pain. However, these treatments are accompanied by several adverse events, including itching, tolerance, dependence, nausea, constipation, sedation, and respiratory depression. Furthermore, the analgesic efficacy of opioids varies among individuals. Therefore, effective pain treatment is often hampered by considerable differences in opioid sensitivity. Insufficient opioid doses can lead to inadequate pain relief, whereas unnecessarily high doses can result in adverse effects, with both commonly observed in clinical settings. Thus, the proper administration of opioids is crucial to meet the needs of individual patients [8]. However, the factors contributing to different inter-individual responses to opioids are not fully understood. Moreover, morphine has been used as a potent analgesic for the treatment of severe chronic pain. However, frequent long-term treatment results in the development of analgesic tolerance. Morphine produces analgesia via its activity at several levels of the nervous system; it inhibits neurotransmitter release from primary afferent terminals in the spinal cord and activates descending inhibitory controls in the midbrain. However, the peripheral action of morphine in regulating pain transmission remains unclear. The endogenous opioid system is activated under pathological conditions. The ratio of morphine-induced partial antinociceptive activity at peripheral sites is more advantageous than that at the central site. Moreover, many researchers have studied the analgesic activity of several opioid receptors for a long period. Despite extensive investigation, the detailed mechanism underlying the analgesic action of morphine is not fully understood..Morphine-induced antinociception is partially reduced in interleukin-31 receptor A -deficient mice, indicating that interleukin-31 receptor A is crucial for morphine-induced peripheral antinociception [8]. Moreover, the repeated administration of interleukin-31 causes itch-associated scratching behavior, which is significantly increased with the increased expression of interleukin-31 receptor A in the dorsal root ganglia. The interaction between cutaneous interleukin-31 and neuronal dorsal root ganglia interleukin-31 receptor A causes severe itch-associated scratching behavior (long-lasting scratching) [9]. Morphine treatment significantly increased the interleukin-31-induced itch-associated scratching behavior) and antinociceptive activity. The subcutaneous injection of morphine induced itch-associated scratching behavior, hygiene behavior, and antinociception. The mechanism of morphine antinociception involves two sites of action: central and peripheral. Previously, it was reported that interleukin-31 may play a more significant role in the modulation of peripheral morphine-induced antinociception via sensory neurons in interleukin-31 receptor A mice than in wild-type mice. Antinociception and scratching behaviors (itch-associated scratching behavior and hygiene behavior) were observed simultaneously during the experimental period. In particular, a close correlation was observed between the development of scratching behavior and morphine-induced antinociceptive action [8]. Postoperative pain occurs following burn excision and/or grafting procedures and is most commonly the result of increased pain from newly created wounds at the skin graft harvesting site. Postoperative pain may occur all over the body, including joints and muscles, head, and limbs, and is accompanied by restlessness, insomnia, sweating or lack of sweating, fatigue, poor appetite, or even dysfunction of the limbs[10].Various mechanisms have been identified for mediation of postoperative pain, which include the role of certain receptors, mediators, and neurotransmitters involved in the peripheral and central sensitization after incision. Poorly managed postoperative pain can lead to complications and prolonged rehabilitation. Uncontrolled acute pain is associated with the development of chronic pain, with a reduction in quality-of-life. Various agents (opioid vs non-opioid), routes (oral, intravenous, neuraxial, regional) and modes (patient-controlled vs as needed) have been tested for the treatment of postoperative pain. However, the clinical utility of these approaches and analgesics is greatly limited by decreased clinical effectiveness and the occurrence of drug-specific side-effects among other factors [10]. The use of medicinal plants is a traditional method of providing relief from illness and can be traced back over five millennia in several civilizations. Over the years, natural products have contributed enormously to the development of important therapeutic drugs used currently in modern medicine. The potential of higher plants as sources for new drugs is still largely unexplored [11]. Natural products have been shown to play an important role in the discovery of analgesic drugs. Different chemical moieties having potent antinociceptive effects have been obtained from natural sources, resulting in novel lead compound classes for designing of analgesic drugs. Among natural compounds, flavonoids are considered important phytochemical classes that displayed distinct pharmacological properties [10]. The current necessity for additional safe analgesic drugs having lessened side-effects can be efficiently covered by investigating both natural and synthetic flavonoids for their pain reducing

properties. The activity of flavonoids does not depend on abolishing a single mechanism but rather reducing varied mechanisms and therefore impacting multiple targets in the neurological processes underlying the expression of pain. Flavonoids are multi-target molecules, and increasing attention has been given to these molecules due to their anti-inflammatory and analgesic properties. Flavonoids have shown potent antinociceptive properties in different animal's models of nociception [10]. To determine the mechanism of flavonoid involved in the central antinociception caused by dichloromethane fraction and apigenin classical antagonists of cholinergic and opioid receptors (atropine, mecamylamine, and naloxone, respectively) and an inhibitor of nitric oxide pathway, was used. The results indicate that there is an involvement of cholinergic receptors since atropine inhibited the antinociceptive effect of dichloromethane fraction and apigenin [11]. A major site of action for cholinomimetics in analgesia is the spinal cord. Painful stimuli are known to increase acetylcholine in the spinal cord. The activation of muscarinic receptors in the spinal cord results in an increased release of inhibitory transmitters along with a decrease in the release on excitatory transmitters, and this in part mediates their antinociceptive effects. Based on this evidence, it is possible that some substances in dichloromethane fraction as well as apigenin activate cholinergic receptors resulting in an antinociceptive effect. The dichloromethane fraction contains apigenin and other compounds. This explains why the treatment with all four antagonists has reversed the antinociceptive effect from dichloromethane fraction and only two of them have inhibited the antinociceptive effect from apigenin [11]. Migraine is a multiphasic neurological disorder associated with multiple symptoms including moderate to severe headache, photophobia, phonophobia, nausea and vomiting. Migraine patients frequently have one or more associated comorbidities including chronic pain disorders, respiratory disorders, cardiovascular and cerebrovascular disorders, psychiatric disorders, and digestive disorders with comorbidities more prevalent among those with chronic migraine as compared to episodic migraine. Patients suffering from migraine frequently report decreased quality of life, decreased work productivity, higher rates of work absences, more use and overuse of both over the counter and prescription medications including opioids, and more healthcare usage including emergency department visits. It was reported that exposure to lights of different wavelengths can affect nociception in rodents. For example, exposure to green light emitting diode resulted in antinociception in naïve rats and antihyperalgesia in rats with neuropathic pain that was dependent on engagement of the visual system. Moreover, green light emitting diode exposure was accompanied by anxiolysis in rats. Given this pain modulating effect of light, in particular the antinociceptive effect green light emitting diode, we hypothesized that green light emitting diode therapy results in reduction of headache-days/month in migraineurs with concomitant improvement in quality-of-life measures [12]. Light has broad effects on biological functions beyond image vision that have been widely demonstrated in human and rodent studies. The regulatory effects of light (including green light) on pain-related behaviors have also been observed in humans with functional pain disorders and pain modelling animals. For example, patients with fibromyalgia exhibit marked aversion to natural light, suggesting that innocuous light is perceived as an aversive or even painful input, whereas exposure to green light was found to alleviate fibromyalgia symptoms. These findings suggest that specific properties of light could function as determining factors in the neurological effects of light on pain perception [13]. The mechanism of the green light emitting diode pain relief remains unknown. It was shown that when we fit rats with clear contact lenses that did not impede or change the wavelength of green light emitting diode, they experienced antinociception when exposed to green light emitting diode. However, when the rats were fitted with opaque contact lenses which did not allow light to pass to the retina, they did not develop antinociception. Therefore, green light emitting diode appears to require the visual system, at least in our rodent studies. It is unclear which part of the visual system might mediate the effects of green light emitting diode. The intrinsically photosensitive retinal ganglion cells project to the midbrain and are involved in triggering photophobia in migraine patients. It is therefore possible that green light emitting diode is mediating its effect through the intrinsically photosensitive retinal ganglion cells [12]. Future studies will be required to elucidate the mechanisms of green light emitting diode and the components of the visual system involved. Other pain modulatory mechanisms may also play a role. For example, in rats green light emitting diode significantly increased the levels of proenkephalin mRNA in the spinal cord and decreased entry of calcium through the voltage-gated N-type calcium channel. It is possible that green light emitting diode produces its effects through several mechanisms acting in harmony. For example, while it has long been established that pain and sleep are intimately related, whether green light emitting diode improved pain which then led to an improvement in sleep or vice versa could not be determined in this study. The effect of light on sleep has been documented previously with blue light having profound effects on melatonin and sleep. Therefore, it is possible that green light emitting diode therapy may have independently improved both sleep and pain by different mechanisms [12]. Although alterations in not only the pain sensitivity but also the analgesic effects of opioids have been reported under conditions of stress, the influence of unpredictable chronic mild stress on the antinociceptive effects of opioid analgesics remains to be fully investigated. Endogenous opioid systems play important roles in the modulation of pain sensitivity and stress responses. On the other hand, various types of stressors have been known to alter not only pain sensitivity but also the analgesic effects of opioids. Several studies have demonstrated the potentiation of the antinociceptive effects of opioid analgesics by acute stress, such as restraint stress and cold-water swim stress, whereas other reports have shown the attenuation of the antinociceptive effect of morphine by chronic stress, such as chronic restraint stress and chronic cold-water swim stress. However, the mechanisms underlying the reduced antinociceptive effect of morphine under conditions of chronic stress remain to be elucidated [14]. Unlike the case of morphine, the antinociceptive effect of tramadol was not reduced under the unpredictable chronic mild stress condition. In the context of previous reports that tramadol has inhibitory effects on noradrenaline and serotonin transporters in addition to its agonistic effect on opioid receptors, this result suggests the important role of inhibitory effects on the transporters in the antinociceptive effect of tramadol under unpredictable chronic mild stress condition. Thus, we examined the effects of pretreatment with noradrenaline and serotonin

transporter inhibitors and found that pretreatment with a noradrenaline reuptake inhibitor but not a serotonin reuptake inhibitor ameliorated the reduced antinociceptive effect of morphine under the unpredictable chronic mild stress condition. Doses of serotonin transporter inhibitor (escitalopram; 1, 3 mg/kg) used in the present study are thought to be sufficient for behavioral experiments [14]. Thus, these results suggest that the reduced antinociceptive effect of morphine under the unpredictable chronic mild stress condition may be due to the downregulation of noradrenergic transmission. In this context, Chen et al. reported that chronic social defeat stress increased the expression of noradrenalin transporter mRNA and protein in the locus coeruleus, which supposedly down-regulated noradrenergic transmission. Additionally, the electrophysiological study conducted by Bravo et al. showed that chronic mild stress induced reduction in noradrenergic transmission in the locus coeruleus.It is thought that chronic pain itself also constitutes chronic stress. It has been reported that chronic pain induces dysfunction in the noradrenergic transmission in the LC of rats in the neuropathic pain model. Combined with a series of previous findings, the present results suggest that compounds having both an agonistic effect on opioid receptors and an inhibitory effect on noradrenaline transporters, or a combination of opioids and noradrenaline reuptake inhibitors, may be effective in the treatment of patients suffering from chronic pain. In support of this notion, antidepressants with an inhibitory effect on noradrenaline transporters (e.g., serotonin–noradrenaline reuptake inhibitors, tricyclic antidepressants) have been reported to be more effective than selective serotonin reuptake inhibitors for the treatment of chronic pain [14]. Lorcaserin is a serotonin 5-HT2C receptor agonist that has recently been reported to reduce abuse-related effects of the opioid analgesic oxycodone. The goal of the studies was to evaluate the effects of adjunctive lorcaserin on opioid-induced analgesic-like behavior using the tail-flick reflex test as a mouse model of acute thermal novice. It was shown that lorcaserin, when injected locally (t.), produced antinociceptive effects in a dose-dependent manner with a peak action after 10 min of injection; and that this effect was opioid receptor-independent. On the contrary, when lorcaserin is injected systemically (s.c.) up to 4 mg/kg, it did not show any significant antinociceptive effect on the tail-flick reflex test. Interestingly, however, lorcaserin (s.c.) potentiated oxycodone-induced antinociception in a dose-dependent manner; a similar adjunctive effect was observed when morphine and fentanyl were administered. Furthermore, administration of lorcaserin (s.c.) did not significantly affect the distribution of oxycodone within the brain or spinal cord [15]. Opioid antinociception is mediated through stimulation of central (periaqueductal gray, nucleus reticularis paragigantocellularis and dorsal horn) and peripheral (afferent nociceptive fibers) mechanisms. It is well established that the serotonin and opioid systems share similar anatomical locations within the periaqueductal gray and the dorsal horn of the spinal cord. Within the dorsal horn, opioid receptors are located both pre- and postsynaptically, preventing transmission of noxious information from Ad and C fibers. Similarly, serotonin receptors are present in primary afferent terminals and interneurons facilitating or inhibiting nociceptive transmission depending on the receptor subtype. For instance, noxious stimuli activate opioidergic neurons in the periaqueductal gray, which in turn modulate serotonergic projections to supraspinal nuclei including the nucleus accumbens and amygdala. In addition, morphine administration increases serotonin in the spinal cord. By contrast, the activation of 5-HT1A receptor in the spinal cord and hypothalamus inhibits the release of endogenous opioids. Moreover, targeting the 5-HT3 receptor can attenuate or enhance morphine-induced analgesia depending on the animal model tested. In the present study, we showed that lorcaserin, a highly selective 5-HT2C receptor agonist, induced a dose-dependent antinociceptive effect when administered locally at the spinal canal, whereas systemic lorcaserin administration did not elicit a significant antinociceptive response [15]. Additionally, we found that activation of the 5-HT2C receptor upon lorcaserin administration augmented opioid-mediated antinociceptive effects. This adjunctive effect of lorcaserin was observed via both parenteral injection (s.c.) and local administration at the spinal canal (i.t.). Early reports proved the effectiveness of 5-HT2C receptor agonism in nicotine, cocaine and alcohol addiction, and a recent study showed that lorcaserin administration inhibits oxycodone intake in rats. The findings presented here have important implications for the development of adjunctive therapies to improve opioid-induced analgesia [15]. Subcutaneous administration of codeine has been advocated for the treatment of postoperative pain in dogs.The mechanism of action of this drug is not fully elucidated. In other species, its analgesic effects appear to be related to its metabolites, such as codeine-6-glucuronide, norcodeine and morphine. Clinical effectiveness or pharmacokinetic profile of codeine has not been reported in the cat. The low efficacy of codeine might be related to its poor bioavailability as observed after oral administration of a similar dose in dogs. Poor bioavailability would explain the lack of antinociception of oral codeine in our study and further pharmacokinetic profile study is warranted to confirm this hypothesis in cats. The seemingly low analgesic efficacy of codeine could be influenced by its metabolism [16]. The metabolites of codeine such as morphine, norcodeine and codeine-6-glucuronide are thought to be responsible for its analgesic effects. Humans produce different amounts of these metabolites owing to polymorphisms within metabolic enzymes, such as cytochrome P450, that mediate opioid metabolism. Ultimately, there is a large variability in analgesic responses, and the same could occur with cats. Besides, one could argue that thermal testing may not be ideal for the evaluation of antinociception after codeine. However, the antinociceptive effects of different opioids have been demonstrated using thermal antinoception in cats, and the model seems to be appropriate for the study of opioid analgesia. The route of administration could affect the pharmacokinetic profiles and pharmacodynamics and therefore the analgesic effects of codeine as observed with buprenorphine in cats. In fact, subcutaneous administration of codeine provides postoperative analgesia in dogs undergoing mandibulectomy or maxilectomy. Dosage regimens for codeine have not been reported in cats. The dose (~2 mg/kg) may not have been appropriate as it was based on a canine study. It is unclear if and how codeine was absorbed by the gastrointestinal tract as no pharmacokinetic profile data are available[16]. Buprenorphine has been widely investigated in cats, using the same antinociceptive model and route of administration. In fact, buprenorphine significantly increased thermal threshold after buccal administration in different studies, and, for this reason, it was chosen to be our positive control treatment. Therefore, it is surprising that thermal antinociception was significantly recorded

at only 3 h when compared with baseline or saline. However, similar findings were observed in a recent study where buccal administration of buprenorphine significantly increased thermal threshold at only one point (44 mins after drug administration) when compared with baseline. Inconsistent thermal antinociception after buprenorphine in this study might be explained by one or more factors, including individual variation in PK data resulting in lower drug bioavailability in some individuals, drug spillage, different formulation of the drug and the first-pass effect. Buccal administration of buprenorphine implies that the drug is absorbed by the oral mucosa into the bloodstream via capillaries bypassing the portal vein [16]. Spironolactone, eplerenone, chlorothiazide and furosemide are diuretics that have been suggested to have antinociceptive properties, for example via mineralocorticoid receptor antagonism. In co-administration, diuretics might enhance the antinociceptive effect of opioids via pharmacodynamic and pharmacokinetic mechanisms. Strong opioids are an established choice for moderate to severe acute and cancer pain. Spironolactone, eplerenone, furosemide and chlorothiazide are diuretics used to treat hypertension and heart failure. Recently, these drugs have also become of interest in the treatment of pain. Spironolactone and eplerenone have been shown to have antinociceptive effects in experimental neuropathic and back pain in the rat. Furosemide has been shown to reduce nociceptive behaviour in experimental inflammatory pain in the rat and chlorothiazide has been shown to have a weak thermal antinociceptive effect of its own. In addition, spironolactone, chlorothiazide and furosemide have been suggested to potentiate the antinociceptive effect of morphine in the rat [17]. It was found that spironolactone increased oxycodone and morphine brain concentrations and antinociception in acute thermal nociceptive tests in the rat. Spironolactone, eplerenone, furosemide or chlorothiazide had no independent effects in acute thermal nociceptive tests. Eplerenone or spironolactone did not show any acute antinociceptive effects in musculocutaneous tissue injury, either. Eplerenone and chlorothiazide did not affect the antinociceptive effect of oxycodone or morphine, while furosemide caused a minor effect. The results are in line with our previous work where spironolactone enhanced the effect of morphine and increased the morphine brain concentrations. The effects of spironolactone, eplerenone, furosemide and chlorothiazide on oxycodone-induced antinociception have not been previously studied. The effect of eplerenone and furosemide on morphine-induced antinociception is also novel [17]. Botulinum neurotoxins have been widely used to treat a variety of clinical ailments associated with pain. The inhibitory action of botulinum neurotoxins on synaptic vesicle fusion blocks the releases of various pain-modulating neurotransmitters, including glutamate, substance P, and calcitonin gene-related peptide, as well as the addition of pain-sensing transmembrane receptors such as transient receptor potential to neuronal plasma membrane. In addition, growing evidence suggests that the analgesic and anti-inflammatory effects of botulinum neurotoxins are mediated through various molecular pathways. Recent studies have revealed that the detailed structural bases of botulinum neurotoxins interact with their cellular receptors and soluble NSF attachment receptors [18]. Along with the expected neuromuscular effects, botulinum neurotoxin A has been reported to reduce the pain associated with hyperactive muscular disorders. Although decreased muscle contractions due to the inhibition of the release of acetylcholine at the neuromuscular junctions may indirectly contribute to pain relief, the low doses botulinum neurotoxin A necessary to affect pain relief, which often persists longer than the accompanying neuroparalytic effects, suggest the neurotoxin's action on pain fibers and sensory, or autonomous nerves. In addition to mitigating pain associated with hyperactive muscle contractions, the antinociceptive action of botulinum neurotoxin A has been reported in various chronic pain associated with migraines and other types of neuropathic disorders. Where and how botulinum neurotoxin A acts in nociception are still largely in debate. The dominant opinion is that botulinum neurotoxin A blocks the exocytosis of synaptic vesicles carrying neurotransmitters or inflammatory mediators, or that of other exocytic vesicles harboring pain sensors in peripheral sensory neurons. The hypothesis of botulinum neurotoxin A's central effects is highly controversial, due to the variability of experimental condition including the dosage of toxin treatment [18]. Nonsteroidal anti-inflammatory drugs are very commonly used, but their adverse effects warrant investigating new therapeutic alternatives. Polyalthic acid, a labdane-type diterpenoid, is known to produce gastroprotection, tracheal smooth muscle relaxation, and antitumoral, antiparasitic and antibacterial activity. For treating pain, nonsteroidal anti-inflammatory drugs are among the most widely used medications. When frequently taken, however, nonsteroidal anti-inflammatory drugs can trigger adverse events, such as gastrointestinal ulcers (with consequent bleeding, perforation and/or obstruction), kidney dysfunction, and cardiovascular events, all entailing the risk of death. Although nonsteroidal anti-inflammatory drugs that are selective cyclooxygenase-2 inhibitors reduce the incidence of gastrointestinal complications, they have been linked to kidney problems and an increased risk of cardiovascular complications [19]. Thus, new types of pain management therapies are needed. Medicinal plants are a possible alternative for treating pain. Indeed, some of the metabolites derived from plants and employed in traditional medicine have provided the basis for discovering and developing modern drug therapy. Labdane-type diterpenes are an excellent example of natural products with analgesic activity. They also have potential antifungal, antibacterial, antimutagenic, cytotoxic and anti-inflammatory effects. Polyalthic acid is a labdane-type diterpenoid known to promote gastroprotection and the relaxation of tracheal smooth muscle cells, as well as having antitumoral, antiparasitic and antibacterial activity. The anti-inflammatory and analgesic potential of polyalthic acid has not yet been described in literature to the best of our knowledge. Hence, the current contribution aimed to evaluate the antinociceptive, antiallodynic, antihyperalgesic and anti-inflammatory effect of polyalthic acid on rats with models of pain and inflammation. For the combined treatment of hyperalgesia with polyalthic acid and naproxen, moreover, the type of drug–drug interaction was analyzed [19]. An evaluation was made of the antinociceptive, antiallodynic, antihyperalgesic and anti-inflammatory effect of polyalthic acid orally administered to female rats. These animals showed a greater inflammation response and a higher pain threshold than male rats in carrageenan-induced inflammation and hyperalgesia, respectively. Regarding evidence of the participation of opioid receptors in the antinociceptive activity of polyalthic acid, an antinociceptive effect of opioids has been observed in

several clinical and experimental studies. The opioid system is closely related to the nitric oxide pathway. The intra-hippocampal CA1 injection of L-NAME or L-arginine prevents the antinociceptive activity of the systemic administration of morphine. Very relevant to the present results, peripheral administration of methylene blue, an inhibitor of guanylyl cyclase, prevents the antihyperalgesic effect induced by the local administration of morphine [19]. The involvement of opioid receptors has also been implicated in ketamine antidepressant effects. Pretreatment with the nonselective opioid receptor antagonist naltrexone blocked improvements in depression scores associated with ketamine infusion therapy in humans, signifying that the acute antidepressant effects of ketamine are at least partially dependent upon opioid receptor activation. Opioid receptor antagonism has also been shown to block the antinociceptive effects of ketamine on the tail-flick test in mice. In this study, ketamine antinociception was also blocked by naltrexone pretreatment but not α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor antagonism. In contrast, (2R,6R)-HNK antinociception was not blocked by naltrexone pretreatment, while α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic receptor antagonism blocked the antinociception, signifying that the drug's use different mechanisms to induce pain reduction effects. These results align with previous data in which naltrexone did not block (2R,6R)-HNK mediated reversal of mechanical allodynia in a model of postoperative pain [20]. Cardiac pain is an index of cardiac ischemia that helps the detection of cardiac hypoxia and adjustment of activity in the sufferer [21]. Cerebral ischemia is a severe neurodegenerative condition that, depending on the area involved in the pathological process, leads to disruption of the cognitive and sensorimotor functions of the brain. Even short-term ischemia leads to profound damage to the brain [22]. Drivers and thresholds of cardiac pain markedly differ in different subjects and can oscillate in the same individual, showing a distinct circadian rhythmicity and clinical picture. In patients with syndrome X or silent ischemia, cardiac pain intensity may cause neurogenic stress that potentiates the cardiac work and intensifies the cardiac hypoxia and discomfort of the patient. The autonomic nervous system regulates cardiac pain sensation in cooperation with vasopressin. Vasopressin is an essential analgesic compound, and it exerts its antinociceptive function through actions in the brain (the periaqueductal gray, caudate nucleus, nucleus raphe magnus), spinal cord, and heart and coronary vessels. Vasopressin acts directly by means of V1 and V2 receptors as well as through multiple interactions with the autonomic nervous system and cardiovascular hormones, in particular, angiotensin II and endothelin. The pain regulatory effects of the autonomic nervous system and vasopressin are significantly impaired in cardiovascular diseases [21]. Typical is extreme variability in the severity, prevalence, localization and prevalence of painful conditions, pronounced fluidity of complaints, repeatedly changing during the day. Neurotic fixation on the slow type of cardialgia is also possible (for example, only burning or distension in the precordial region) [23].

**Conclusions:**

Pain has been defined by the International Association for the Study of Pain in an interesting and all-encompassing manner. The International Association for the Study of Pain refers to two dimensions of pain, the physical sensation of intensity and the emotional experience of unpleasantness. Moreover, their definition of pain includes its relation to real or potential tissue damage. The two types of pain identified, acute and chronic, are distinguished by their temporal relationship to an injury or other tissue damage. Acute pain is expected to be of limited duration, while chronic pain persists for at least 3 months. Chronic pain is classified as nociceptive, neuropathic, or nociplastic. Acute pain is managed pharmacologically by means of nonselective non-steroidal anti-inflammatory drugs (NSAIDs), selective cyclooxygenase-2 inhibitors (COX-2), opioids, or a combination of drugs (e.g., an NSAID plus an opioid). Similar treatment options are employed for patients with chronic nociceptive pain. Chronic neuropathic pain is handled with opioids plus paracetamol, followed by drugs that act on the central nervous system (e.g., antidepressants and antiepileptic drugs) [7]. C18 5-HT's mechanism of action appears to involve, at least in part, the activation of the opioid, serotoninergic and cannabinoid systems and has anti-hyperalgesic effects, as observed in the thermal hyperalgesia model. According to these results, this compound may be a novel prototype for future analgesic drugs [5]. Itch and pain are common senses caused by several drugs and physical stimulations. Scratching behavior in mice is divided into two types, itch-associated scratching and hygiene behavior. Moreover, morphine is one of the few drugs that induce itch-associated scratching. In interleukin-31 receptor A mice, itch-associated scratching disappeared upon the administration of morphine, accompanied by the partial disappearance of antinociceptive action. Moreover, we found that interleukin-31 was partially involved in the peripheral analgesic mechanism and that interleukin-31-induced alloknesis inhibited pain. The antinociceptive activity of interleukin-31 was approximately as effective as that of loxoprofen [9]. The antinociceptive effects of morphine but not tramadol was reduced under the unpredictable chronic mild stress condition. Pretreatment with a noradrenaline transporter inhibitor but not a serotonin transporter inhibitor ameliorated the reduced antinociceptive effect of morphine under the unpredictable chronic mild stresscondition. These results suggest that activation of the noradrenergic but not the serotonergic system may ameliorate the reduced antinociceptive effect of morphine under conditions of chronic stress [14]. Drugs targeting both serotonin and noradrenaline transporters such as duloxetine have proven efficacy and are recommended in neuropathic pain. On the contrary, selective serotonin transporter inhibitors have shown a modest clinical analgesic effect and thus are not indicated in pain management. However, it is well established that serotonin receptors are involved in modulating nociceptive processing along the neuroaxis including spinal afferences and descending modulatory pathways from more complex neural circuits within the central nervous system. A reason that can explain this apparent paradox lies in the fact that different serotonin receptors, such as 5-HT2A and 5-HT2C, can exert opposite actions despite their similar cellular distributions and signaling pathways downstream. Thus, their concomitant activation by the endogenous ligand serotonin may blur the contribution of each receptor to serotonin-dependent transmission [15]. The widely reported antinociceptive effect of botulinum neurotoxin A was thought to be primarily mediated by the blocking of neurotransmitter and inflammatory substance release, and the inhibition of plasma membrane insertion of pain sensors at peripheral level. However, observations of bilateral

action in distant regions after unilateral injection of botulinum neurotoxin A suggest the hypothesis that peripherally administered botulinum neurotoxin A spread to central region via axonal transport to target neurotransmission of pain sensory circuits [18].

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