EVALUATION OF ANTI-NOCICEPTIVE AND ANALGESIC ACTIVITIES OF NIZATIDINE IN MICE

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Submitted by

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EVALUATION CERTIFICATE

This is to certify that the dissertation work entitled **"EVALUATION OF ANTI-NOCICEPTIVE AND ANALGESIC ACTIVITIES OF NIZATIDINE IN MICE"** submitted by the student bearing **[REG. No. 261625207]** to **"The Tamil Nadu Dr. M.G.R. Medical University"**, Chennai, in partial fulfillment for the award of Degree of **Master of Pharmacy** in **Pharmacology** was evaluated by us during the examination held on……..…….

Internal Examiner **External Examiner**

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DECLARATION

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I further declare that this work is original and this dissertation has not been submitted previously for the award of any other degree, diploma, associate ship and fellowship or any other similar title. The information furnished in this dissertation is genuine to the best of my knowledge.

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INDEX

ABBREVIATIONS

Abbreviations	Expansion
ANOVA	Analysis of variance
VTA	Ventral tegmental area
PTSD	Post-traumatic stress disorder
CSF	Cerebrospinal fluid
PET	Positron emission tomography
OCD	Obsessive-Compulsive Disorder
PPD	Post-partum depression
COPD	Chronic obstructive pulmonary disease
HVA	Homovanillic acid
CRP	C-reactive protein
TNF-α.	Tumor necrosis factor alpha
HPA	Hypothalamic-pituitary- adrenal axis
SSRI	Selective serotonin reuptake inhibitors
SNRI	Serotonin norepinephrine reuptake inhibitor
MAO-I	Mono amino oxidase inhibitors
GABA	Gamma amino butyric acid
BDNF	Brain derived neurotropic factor
MAPK	Mitogen-activated protein kinase
CREB	Cyclic AMP response element Binding protein
AVP	Arginine vasopressin

INTRODUCTION

The International Association for the Study of Pain defines pain as 'An unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage'. It is considered as a major symptom of various diseases that persists to produce severe physical and psychological distress for many patients by disrupting their quality of life[1]

Various types of pain are classified as follows[2]

- Acute physiological nociceptive pain-Pain elicited by application of an acute noxious stimulus to normal tissue.
- Pathophysiological nociceptive pain-occurs when the tissue is inflamed or injured.
- Spontaneous pain-It is pain in the absence of any intentional stimulation or as hyperalgesia and/or allodynia

Hyperalgesia is extreme pain intensity felt upon noxious stimulation, and allodynia is the sensation of pain elicited by stimuli that are normally below pain threshold.

- Neuropathic pain-results from injury or disease of neurons in the peripheral or central nervous system.

Nociception an overview

Nociception is the encoding and processing of noxious stimuli in the nervous system that can be measured with electrophysiological techniques. Neurons involved in nociception form the nociceptive system. Noxious stimuli activate primary nociceptive neurons with “free nerve endings” (Aδ and C fibres, nociceptors) in the peripheral nerve.Most of the nociceptors respond to noxious mechanical (e.g. squeezing the tissue), thermal (heat or cold), and chemical stimuli and are thus polymodal. Nociceptors can also exert efferent functions in the tissue by releasing neuropeptides [substance P (SP), calcitonin gene-related peptide (CGRP)] from their sensory endings. Thereby they induce vasodilatation, plasma extravasation, attraction of macrophages or degranulation of mast cells, etc. This inflammation is called neurogenic inflammation. Nociceptors project to the spinal cord and form synapses with second order neurons in the grey matter of the dorsal horn. A proportion of second-order neurons have ascending axons and project to the brain stem or to the thalamocortical system that produces the conscious pain response upon noxious stimulation. Other spinal cord neurons are involved in nociceptive motor reflexes, more complex motor behaviour such as avoidance of movements, and the generation of autonomic reflexes that are elicited by noxious stimuli. Descending tracts reduce or facilitate the spinal nociceptive processing. The descending tracts are formed by pathways that originate from brainstem nuclei and descend in the dorsolateral funiculus of the spinal cord. Descending inhibition is part of intrinsic antinociceptive system.

Neurochemistry of Pain

Nociception is mediated by the function of numerous intra- and extra-cellular molecular messengers involved in signal transduction in the peripheral and central nervous systems. All nociceptors, when activated by the requisite mechanical, thermal, or chemical stimulus, transmit information via the excitatory neurotransmitter glutamate[3]

In addition, inflammatory mediators are secreted at site of the original injury to stimulate nociceptor activation. This "inflammatory soup" is comprised of chemicals such as peptides (e.g.,bradykinin), neurotransmitters (e.g., serotonin), lipids (e.g., prostaglandins), and neurotrophins (e.g., NGF). The presence of these molecules excites nociceptors or lowers their activation threshold, resulting in the transmission of afferent signals to the dorsal hornof the spinal cord as well as initiating neurogenic inflammation.3

Neurogenic inflammationis the process by which active nociceptors release neurotransmitters such as substance Pfrom the peripheral terminal to induce vasodilation, leak proteins and fluids into the extracellular space near the terminal end of the nociceptor, and stimulate immune cells which contribute to the inflammatory soup. As a result of these neurochemical changes in the local environment of nociceptors, the activation of Aδ and C fibers increases, and peripheral sensitization occurs[4].

In turn, nociceptive signal transduction up the spinothalamic tract results in elevated release of norepinephrine from the locus coeruleus neurons projecting to thalamus, which in turn relays nociceptive information to somatosensory cortex,

hypothalamus, and hippocampus[5]. As such, norepinephrine modulates the "gain" of nociceptive information as it is relayed for processing in other cortical and subcortical brain regions. Concomitantly, opioid receptors in the peripheral and central nervous systems (e.g., those in neurons of the dorsal horn of the spine and the periaqueductal grey in the brain) result in inhibition of pain processing and analgesia when stimulated by opiates or endogenous opioids like endorphin, enkephalin, or dynorphin.[6] The secretion of endogenous opioids is largely governed by the descending modulatory pain system[7]. The neurotransmitter GABA is also involved in the central modulation of pain processing, by augmenting descending inhibition of spinal nociceptive neurons. A host of other neurochemicals are also involved in pain perception; the neurochemistry of nociception and central-peripheral pain modulation is extremely complex [8].

Descending central modulation of pain

The brain does not passively receive pain information from the body, but instead actively regulates sensory transmission by exerting influences on the spinal dorsal horn via descending projections from the medulla.[9] In their seminal Gate Control theory of pain, Melzack and Wall proposed that the substantia gelatinosa of the dorsal horn gates the perception of noxious stimuli by integrating upstream afferent signals from the peripheral nervous system with downstream modulation from the brain.[10] Interneurons in the dorsal horn can inhibit and potentiate impulses ascending to higher brain centers, and thus they provide a site where the central nervous system controls impulse transmission into consciousness.

The descending pain modulatory system exerts influences on nociceptive input from the spinal cord. This network of cortical, subcortical, and brainstem structures includes prefrontal cortex, anterior cingulate cortex, insula, amygdala, hypothalamus, periaqueductal grey, rostral ventromedial medulla, and dorsolateral pons/tegmentum[11].

The coordinated activity of these brain structures modulates nociceptive signals via descending projections to the spinal dorsal horn. By virtue of the somatotopic organization of these descending connections, the central nervous system can selectively control signal transmission from specific parts of the body. The descending pain modulatory system has both anti- and pro-nociceptive effects. Classically, the descending pain modulatory system has been construed as the means by which the central nervous system inhibits nociceptive signals at the spinal outputs.[12] In acrucial early demonstration, Reynolds observed that direct electrical stimulation of the periaqueductal grey could produce dramatic analgesic effects as evidenced by the ability to undergo major surgery without pain.[13] Yet, this brain system can also facilitate nociception. For instance, projections from the periaqueductal grey to the rostral ventromedial medulla have been shown to enhance spinal transmission of nociceptive information from peripheral nociceptors.[14]

Central modulation of pain may have been a conserved across human evolution due to its potentially adaptive effects on survival. For instance, in situations of serious mortal threat (for example, in the face of war and civil accidents, or more primordially, when being attacked by a vicious animal), suppression of pain might enable a severely-injured individual to continue intense physical activity such as fleeing from danger or fighting a deadly opponent. Yet, the

neurobiological linkages between the brain, the spinothalamic tract, the dorsal horn, and the peripheral nerves also provide a physiological pathway by which negative emotions and stress can amplify and prolong pain, causing functional interference and considerable suffering.

Cognitive, Affective, Psychophysiological, and Behavioral Processes in Pain Perception and Regulation

In addition to the somatosensory elements of pain-processing described above, cognitive and emotional factors are implicit within the definition of pain offered by the International Association for the Study of Pain. Pain perception involves a number of psychological processes, including attentional orienting to the painful sensation and its source, cognitive appraisal of the meaning of the sensation, and the subsequent emotional, psychophysiological, and behavioral reaction, which then feedback to influence pain perception. Each of these processes will be detailed below.

Attention to pain

In the brain, attention allows salient subsets of data to gain preeminence in the competitive processing of neural networks at the expense of other subsets of data.[14] The goal-relevance of a stimulus guides attention to select and distinguish it from the environmental matrix in which it is embedded.[15] Thus, attended stimuli receive preferential information processing and are likely to govern behavior. In this sense, attention allows for the evaluation of salientstimuli, and facilitates execution of approach behaviors in response to appetitive stimuli or avoidance behaviors in response to aversive ones. Thus, depending on its salience to the survival of the

organism, the object of attention elicits the motivation to approach or avoid, while the resultant emotional state, as the manifestation of approach or avoidance motivations, tunes and directs attention.[17,18] By virtue of its significance for health and well-being, pain automatically and involuntarily attracts attention.[19] Yet pain experience varies according to the locus of attention; when attention is focused on pain, it is perceived as more intense,[20] and whereas when attention is distracted from pain, it is perceived as less intense.[21]

Attentional modulation of pain experience correlates with changes in activation of the pain neuromatrix; for instance, attentional distraction reduces pain-related activations in somatosensory cortices, thalamus, and insula, among other brain regions.[22]

Concomitantly, distraction results in strong brain activations in prefrontal cortex, anterior cingulate cortex, and periaqueductal grey, suggesting an overlap and interaction between brain systems involved in attentional modulation of pain and the descending pain modulatory system.[23] In contrast, attentional hypervigilance for pain, a high degree of monitoring internal and external stimuli that is often observed among persons with chronic pain,[24] amplifies pain intensity and is associated with the interpretation of harmless sensations (like moderate levels of pressure) as painfully unpleasant.[25, 26]

Cognitive appraisal of pain

Pain involves a process of cognitive appraisal, whereby the individual consciously or unconsciously evaluates the meaning of sensory signals emanating from the body to determine the extent to which they signify the presence of an actual

or potential harm. This evaluation is decidedly subjective. For instance, experienced weightlifters or runners typically construe the "burn" they feels in their muscles as pleasurable and indicative of increasing strength and endurance; in contrast, a novice might view the same sensation as signaling that damage had occurred. The inherent variability of cognitive appraisal of pain may stem from the neurobiological dissociation between the sensory and affective aspects of the pain experience; change in pain intensity results in altered activation of somatosensory cortex, whereas change in pain unpleasantness results in altered activation of the anterior cingulate cortex.[27,28] Thus, a sensory signal originating from the muscles of lower back might be perceived as a warmth and tightness, or viewed as a terrible agony, in spite of the stimulus intensity being held constant. The manner in which the bodily sensation is appraised may in turn influence whether it is experienced as unpleasant pain or not.[29]

The extent to which a given bodily sensation is interpreted as threatening is in part dependent on whether or not the individual believes he or she is able to cope with that sensation. If, during this complex cognitive process of appraisal, available coping resources are deemed sufficient to deal with the sensation, then pain can be perceived as controllable.

Pain intensity is reduced when pain is perceived to be controllable, whether or not the individual acts to control the pain. Ventrolateral prefrontal cortex activation is positively associated with the extent to which pain is viewed as controllable and negatively correlated with subjective pain intensity. This brain region is implicated in emotion regulation efforts, such as when threatening stimuli are reappraised to be benign.[30, 31] Concomitantly, reinterpreting pain as a harmless

sensation (e.g., warmth or tightness) predicts higher perceived control over pain,[32] and psychological interventions have been shown to reduce pain severity by increasing reinterpretation of pain sensations as innocuous sensory information.[33] In contrast, pain catastrophizing (i.e., viewing pain as overwhelming and uncontrollable) is associated with greater pain intensity irrespective of the extent of physical impairment[34] and prospectively predicts the development of low back pain.[35]

Emotional and psychophysiological reactions to pain

The aversive nature of pain elicits a powerful emotional reaction that feeds back to modulate pain perception. Pain often results in feelings of anger, sadness, and fear depending on the how the pain is cognitively appraised. For instance, the belief "It's not fair that I have to live with this pain" is likely to lead to anger, whereas the belief "My life is hopeless now that I have this pain" will likely result in sadness. Fear is a common reaction to pain when individuals interpret the sensations from the body as indicating the presence of serious threat.

These emotions are coupled with autonomic, endocrine, and immune responses which may amplify pain through a number of psychophysiological pathways. For example, pain induction significantly elevates sympathetic nervous system activity, marked by increased anxiety, heart rate, and galvanic skin response.[36] Furthermore, negative emotions and stress increase contraction of muscle tissue; elevated electromyographic activity occurs in the muscles of the back and neck under conditions of stress and negative affect and is perceived as painful spasms.[37, 38] This sympathoexcitatory reaction coupled with emotions like anger and

fear may reflect an evolutionarily conserved, active coping response to escape the painful stimulus. Yet negative emotional states intensify pain intensity, pain unpleasantness, and pain-induced cardiovascular autonomic responses, while reducing the sense of perceived control over pain.[39] Stress and negative emotions like anger and fear may temporarily dampen pain via norepinephrine release, but when the sympathetic "fight or flight" response is prolonged it can increase blood flow to the muscle and increase muscle tension which may aggravate the original injury.[40] Alternatively, pain inputs from the viscera and muscles may stimulate cardiac vagal premotor neurons, leading to hypotension, bradycardia, and hyporeactivity to the environment – a pattern of autonomic response that corresponds with passive pain coping and depressed affect.[41] In addition to autonomic reactivity, proinflammatory cytokines and the stress hormone cortisol are released during the experience of negative emotion; these bio-molecular factors enhance nociception, facilitate processing of aversive information in the brain, and when their release is chronic or recurrent, may cause or exacerbate tissue damage.[42,43]

Moreover, negative emotions are associated with increased activation in the amygdala, anterior cingulate cortex, and anterior insula – these brain structures not only mediate the processing of emotions, but are also important nodes of the pain neuromatrix that tune attention toward pain, intensify pain unpleasantness, and amplify interoception (the sense of the physical condition of the body).[44,45] Thus, when individuals experience negative emotions like anger or fear as a result of pain or other emotionally salient stimuli, the heightened neural processing of threat in affective brain circuits primes the subsequent perception of pain [46,47] and increases

the likelihood that sensations from within the body will be interpreted as painful.[47-49] The fear of pain, a clinical feature of chronic pain patients, is associated with hypervigilance for and sustained attention to pain-related stimuli.[50] Thus, negative emotions bias attention toward pain, which then increase its unpleasantness. In addition, negative emotions and stress impair prefrontal cortex function, which may reduce the ability to regulate pain using higher order cognitive strategies like reappraisal or viewing the pain as controllable and surmountable.[51,52] Thus, anger, sadness, and fear may result from acute or chronic pain and in turn feedback into the bio-behavioral processes that influence pain perception to exacerbate anguish and suffering.

Behavioral reactions to pain

Pain is not only a sensory, cognitive, and emotional experience, but also involves behavioral reactions that may alleviate, exacerbate, or prolong pain experience. Typical pain behaviors in low back pain include grimacing, rubbing, bracing, guarded movement, and sighing.[53]

These behaviors facilitate the communication of pain and exert social influences that may have vicarious gain for the individual suffering from pain; such benefits include sympathy, acts of kindness and generosity, tolerance, lowered expectations, and social bonding, among others.[54] In addition, guarding or avoidance of activities associated with pain may be negatively reinforcing by virtue of the temporary alleviation of pain experience.[55] The fact that these avoidant behaviors decrease the occurrence of pain results in increasing use of avoidance as a coping strategy. Yet, greater use of avoidance as a result of fear of pain predicts higher

levels of functional disability.[56] It is not merely that persons with greater pain-related disability engage in more avoidant behaviors, but rather studies indicate that avoidant behavior and beliefs are a precursor to disability.[57–59] Avoidance contributes to negative clinical outcomes in patients with chronic low back pain. Fear-avoidance of pain influences physical impairment and is more strongly associated with functional disability than pain severity.[60,61] In contrast, progressive increase in activity through exercise has been shown to result in significant benefits in pain, disability, physical impairment, and psychological distress for low back pain patients.69 In light of the robust relation between coping behaviors and pain, behavioral and psychosocial interventions hold great promise in reducing pain intensity and pain-related functional disability in chronic pain conditions such as low back pain.

Animal models for screening antinociceptive activity

Acetic acid induced writhing in mice is simple and most reliable inflammatory pain model widely used for the evaluation of peripheral analgesics. The pain caused by acetic acid is said to be an inflammatory pain due to increase in the capillary permeability and release of endogenous mediators such as PGE1, PGE2, histamine, bradykinin, substance P etc… which sensitize the nociceptive nerve endings[62]. NSAIDs are known to inhibit the COX enzyme in the peripheral tissues which is responsible for the production of pain mediators.

In the tail immersion test, animal's tail is immersed in hot water which provokes an abrupt movement of the tail and sometimes the recoiling of the whole body and the reaction time is monitored[63]. Immersion of the tail in a hot liquid

increases its temperature very quickly and in a more or less linear fashion, which is different from radiant heat.

Hot plate test consists of introducing a rat or mouse into an open-ended cylindrical space with a floor consisting of a metallic plate that is heated by a thermode or a boiling liquid[64]. A plate heated to a constant temperature produces two behavioral components that can be measured in terms of their reaction times, namely paw licking and jumping. Both are considered to be supraspinally integrated responses. As far as analgesic substances are concerned, the paw licking behavior is affected only by opioids. On the other hand, the jumping reaction time is increased equally by less powerful analgesics such as acetylsalicylic acid or paracetamol, especially when the temperature of the plate is 50°C or less[65] or if the temperature is increased in a progressive and linear fashion, e.g., from 43 to 52°C at 2.5°C/min[66]. The specificity and sensitivity of the test can be increased by measuring the reaction time of the first evoked behavior regardless of whether it is paw-licking or jumping or by lowering the temperature[67].

Formalin test model is useful in evaluating the anti-nociceptive activity in two different phases. In the initial phase, direct chemical stimulation of the sensory afferent nerve ending particularly C fibers causes neurogenic pain. In the later phase, induction of inflammatory pain occurs due to the increased production and/or action of various inflammatory mediators. Centrally acting analgesics such as morphine effectively reduce or prevent the paw licking in both the phases whereas, peripheral analgesics such as diclofenac reduce paw licking only in late phase due to inflammatory pain[68].

2. LITERATURE REVIEW

Netti *et al.,* [69] studied central effects of histamine H2-receptor agonists and antagonists on nociception in the rat. The effects of intracerebroventricular injection of histamine H2-receptornagonists (4-methylhistamine, 4-MeH; dimaprit, DIM), H2-antagonists (cimetidine, CIM; ranitidine, RAN; famotidine, FAM) and of the DIM chemical analogue SK&F 91487 on hot-plate latency in rats were examined. Both DIM (0.4-0.8 mumol/rat) and 4-MeH (0.4-0.8 mumol/rat) significantly enhanced the pain threshold, whereas, SF&F 91487 (0.8 mumol/rat) had no effect, indicating that DIM antinociception is specifically due to its activity on histamine (HA) receptors. The H2-antagonists CIM (0.8 mumol/rat) and RAN (0.6 mumol/rat) also enhanced the pain threshold, while FAM (0.03 mumol/rat) did not modify pain latency. When injected before 4-MeH, FAM reduced the antinociceptive effect of 4-MeH. These findings suggest that the antinociceptive activity of CIM and RAN is not related to specific blockade of H2-receptors and that the activation of HA-H2-receptors is inhibitoryto nociception.

Ahmadi *et al.,* [70] studied Hepatoprotective, antinociceptive and antioxidant activities of cimetidine, ranitidine and famotidine as histamine H2 receptor antagonists. Antinociceptive effects were, determined using the hot plate test in mice. All compounds also showed a dose-dependent and marked analgesic activity in mice relative to controls.

Bethesda *et al.,* [71]studied the hepatotoxicity of nizatidine and he reported that Nizatidine has been linked to rare instances of clinically apparent acute liver injury. The selective histamine type 2 receptor antagonists/blockers (H2 blockers)

are widely used in the treatment of acid-peptic disease, including duodenal and gastric ulcers, gastroesophageal reflux disease and common heartburn. The four H2 blockers in current use are available by prescription as well as over-the-counter, and are some of the most widely used drugs in medicine. The H2 blockers are very well tolerated, but have been linked to rare instances of clinically apparent liver injury. The H2 receptor blockers act by binding to histamine type 2 receptors on the basolateral (antiluminal) surface of gastric parietal cells, interfering with pathways of gastric acid production and secretion. The selectivity of H2 blockers is of key importance, as they have little or no effect on the histamine type 1 receptors, which are blocked by typical antihistamines that are used to treat allergic reactions and have little effect on gastric acid production. The selective H2 blockers are less potent in inhibiting acid production than the proton pump inhibitors (which block the common, final step in acid secretion) but, nevertheless, suppress 24 hour gastric acid secretion by about 70%. The effect of H2 blockers is largely on basal and nocturnal acid secretion, which is important in peptic ulcer healing. The selective H2 blockers were first developed in the early 1990s by Sir James Black, who subsequently received the Nobel Prize for his work developing selective receptor antagonists for clinical use (including the beta blockers as well as the H2 blockers). The initial H2 blocker approved for use in the United States was cimetidine (1977), which was followed by ranitidine (1983), famotidine (1986), and nizatidine (1988). All four of these agents are available by prescription and as over-the-counter oral formulations. Intravenous and intramuscular forms are available for cimetidine, ranitidine and famotidine. The four H2 receptor blockers available in the United States have similar spectra of activity, side effects and clinical indications. These medications are extremely well tolerated and are used by a high proportion of the

general population to treat peptic ulcer disease, heartburn, esophagitis, and miscellaneous minor upper gastrointestinal symptoms. Their listed indications are for treatment of gastric and duodenal ulcer and esophageal reflux disease, and to prevent stress ulcers. Side effects are uncommon, usually minor and include diarrhea, constipation, fatigue, drowsiness, headache and muscle aches. The H2 receptor blockers are metabolized in the liver by the cytochrome P450 system. Among the four agents, cimetidine is distinctive in its potent inhibition of the P450 system (CYP 1A2, 2C9 and 2D6), which can result in significant drug interactions. All four H2 receptor blockers have been implicated in rare cases of clinically apparent, acute liver injury. The most cases have been linked to ranitidine and cimetidine, but these two agents are also the most commonly used.

Sanad et al.,[72] studied the Radioiodination and biological evaluation of nizatidine as a new highly selective radiotracer for peptic ulcer disorder detection. Nizatidine has been labeled using [125 I] with chloramine-T as oxidizing agent. Factors such as the amount of oxidizing agent, amount of substrate, pH, reaction temperature, and reaction time have been systematically studied to optimize the iodination. Biodistribution studies indicate the suitability of radioiodinated nizatidine as a novel tracer to image stomach ulcer. Radioiodinated nizatidine may be considered a highly selective radiotracer for peptic ulcer imaging.

Yamaji *et al.,*[73] studied the effects of successive doses of nizatidine, cimetidine and ranitidine on serum gastrin level and gastric acid secretion. Nizatidine (N-[2-[[[2-[(dimethylamino)methyl]- 4-thiazolyl]methyl]thio]ethyl]-N'-methyl-2-nitro-1,1-ethenediamine, CAS 76963-41-2) is a new histamine H2-receptor antagonist which shows suppression of gastric acid secretion and antiulcer activity.

In the present experiment, the effects of single s.c. administration of nizatidine, cimetidine and ranitidine on serum gastrin levels were studied in fasted rats. Nizatidine at 100 mg/kg increased serum gastrin level 3 h after administration, which however, returned to basal level 6 h after administration. Cimetidine and ranitidine at respective doses of 250 and 100 mg/kg markedly increased serum gastrin levels 3 and 6 h after administration. In a previous study, the suppressive effect of nizatidine on basal gastric acid secretion was 82.8% at a dose of 100 mg/kg s.c. in rat pylrus-ligated model. On the basis of these findings, changes in basal gastric acid secretion and serum gastrin level after withdrawal of nizatidine, cimetidine and ranitidine administered for 14 consecutive days were studied. One day after withdrawal, nizatidine at 100 mg/kg showed a tendency to increase the basal gastric acid secretion. However, 3 and 7 days after administration, almost no changes were obtained. Cimetidine at 250 mg/kg showed a tendency to increase the basal gastric acid secretion 7 days after withdrawal of the drug. Ranitidine at 100 mg/kg induced no changes in basal gastric acid secretion after withdrawal. No obvious influences of all drugs on serum gastrin level after withdrawals were obtained. These results indicate that consecutive administration of nizatidine may cause only a transient increase of gastric acid secretion but no hypergastrinaemia after its withdrawal.

Probst *et al.*,[74] studied the Preclinical toxicology studies with nizatidine, a new H2-receptor ntagonist: acute, subchronic, and chronic toxicity evaluations. Nizatidine (NIZ), a new antiulcer drug, was evaluated for toxicity in acute, subchronic, and chronic tests. Acute toxicity studies were conducted in rats, mice, dogs, and monkeys. Median lethal doses (MLD) in rodents were greater than 1600,

230, and 1000 mg/kg by oral (po), iv, and sc administration, respectively. No deaths occurred in dogs given single doses of 800 mg/kg (po), 75 mg/kg (iv), or 225 mg/kg (im) or in monkeys given 1200 mg/kg (po) or 200 mg/kg (iv). Rats survived up to 1.0% dietary NIZ (daily intake ranging from 24 to 800 mg/kg/day) for 1 year. Slight decreases in body weight gain and increases in liver and kidney weights occurred. Slight decreases in erythrocytic parameters at 3 months were not present at 6 or 12 months. Mice survived up to 1.5% dietary NIZ for 3 months and effects were limited to slight decreases in body weight gain and increases in relative liver weight. Dogs survived oral doses up to 800 mg/kg/day for 3 months but had numerous clinical signs of toxicity and body weight loss. All dogs given oral NIZ doses up to 400 mg/kg/day survived except for one high-dose dog that was killed in a moribund condition following convulsions in the 41st week of treatment. Effects in dogs included miosis, body weight loss, increased thrombocyte counts, and decreased hepatic microsomal enzyme activity and P450 content. The increase in thrombocyte counts was unaccompanied by changes in thrombocyte function and did not reoccur in a subsequent study. A decrease in plasma testosterone in two of three surviving male dogs given 400 mg/kg/day for 1 year was unaccompanied by effects on the size or morphology of testes or prostate. Peak plasma levels of NIZ in all species tested were in excess of human plasma levels after therapeutic doses. In conclusion, there was no evidence of significant toxicity in organs or tissues including those sites (gastric mucosa, male sex organs, and liver) that have been affected by some agents of this therapeutic class.

Kim *et al.,* [75] studied Histamine Receptor Antagonists, Loratadine and Azelastine, Sensitize P-gp-overexpressing Antimitotic Drug-resistant KBV20C

Cells Through Different Molecular Mechanisms. These findings provide important information regarding the sensitization of drug-resistant cells and indicate that loratadine may be used in patients with potentially resistant cancer without any toxic effects from P-gp inhibition.

Liu *et al.,*[76] studied the Gastroprotective effects of several H2RAs on ibuprofen-induced gastric ulcer in rats. Preliminary screening of literature with the criteria of low toxicity led to four histamine-2 receptor antagonists (H2RAs): nizatidine, famotidine, lafutidine, and roxatidine acetate, which were selected for further investigation. These drugs were evaluated systemically by examining the gastric ulcer index, lipid peroxidation (LPO), membrane permeability, toxicity to main organs, and the influence on the activity of antioxidant enzymes, and myeloperoxidase (MPO). Nizatidine was found to be the best gastric protective agent. It exhibited excellent protective effect by increasing antioxidant enzyme activity, decreasing MPO activity, reducing LPO, and membrane permeability. Combination treatment with nizatidine and ibuprofen did not show any significant toxicity. Nizatidine was considered as a good option for combination therapy with ibuprofen especially for diseases that require long-term treatment such as arthritis and osteoarthritis.

Morrissey *et al.,*[77] studied the effect of Nizatidine, a MATE2K Selective Inhibitor, on the Pharmacokinetics and Pharmacodynamics of Metformin in Healthy Volunteers. This study demonstrates that a selective inhibition of MATE2K by nizatidine affected the apparent volume of distribution, tissue concentrations, and peripheral effects of metformin. However, nizatidine did not alter systemic

concentrations or the CLR of metformin, suggesting that specific MATE2K inhibition may not be sufficient to cause renal DDIs with metformin.

Dahan *et al.,*[78] studied the H2 receptor antagonist nizatidine is a P-glycoprotein substrate: characterization of its intestinal epithelial cell efflux transport. The intestinal epithelial efflux transport mechanisms of nizatidine were investigated and characterized across Caco-2 cell monolayers, in the concentration range 0.05-10 mM in both apical-basolateral (AP-BL) and BL-AP directions, and the transport constants of P-glycoprotein (P-gp) efflux activity were calculated. The concentration-dependent effects of various P-gp (verapamil, quinidine, erythromycin, ketoconazole, and cyclosporine A), multidrug resistant-associated protein 2 (MRP2; MK-571, probenecid, indomethacin, and p-aminohipuric acid), and breast cancer resistance protein (BCRP; Fumitremorgin C) inhibitors on nizatidine bidirectional transport were examined. Nizatidine exhibited 7.7-fold higher BL-AP than AP-BL Caco-2 permeability, indicative of net mucosal secretion. All P-gp inhibitors investigated displayed concentration-dependent inhibition on nizatidine secretion in both directions. The IC(50) of verapamil on nizatidine P-gp secretion was 1.2 x 10(-2) mM. In the absence of inhibitors, nizatidine displayed concentration-dependent secretion, with one saturable (J(max) = 5.7 x 10(-3) nmol cm(-2) s(-1) and K(m) = 2.2 mM) and one nonsaturable component (K(d) = 7 x 10(-4) microL cm(-2) s(-1)). Under complete P-gp inhibition, nizatidine exhibited linear secretory flux, with a slope similar to the nonsaturable component. V(max) and K(m) estimated for nizatidine P-gp-mediated secretion were 4 x 10(-3) nmol cm(-2) s(-1) and 1.2 mM, respectively. No effect was obtained with the MRP2 or the BCRP inhibitors. Being a drug commonly used in pediatrics, adults, and elderly,

nizatidine susceptibility to efflux transport by P-gp revealed in this paper may be of significance in its absorption, distribution, and clearance, as well as possible drug-drug interactions.

Tomokane *et al.,* [79] studied the clinical study on the effects of nizatidine on gastric motility and cardiac autonomic function. Investigations using electrogastrography and spectral analysis of heart rate variability. In this, two protocols were adopted to study nizatidine's effects on cardiac autonomic function and gastric motility. Protocol I--Acute: "Group C-I": 10 healthy volunteers received a single oral dose of nizatidine 150 mg. Protocol II--Chronic: "Group DM without N": 15 patients with diabetes mellitus (DM) were observed prior to administration of nizatidine. "Group DM with N": The same 15 patients with DM received nizatidine 300 mg/day for more than 30 days. "Group C-II": This control group was composed of 15 healthy volunteers not receiving nizatidine. In all groups, EGGs were recorded before and after a meal, and autonomic nervous function and QT interval of ECG dispersions were simultaneously evaluated. In Group C-I, nizatidine significantly increased the peak power amplitude of 3 cycles/min (cpm) frequency, but did not significantly change the dominant frequency of the 3-cpm waves. In Group DM with N, nizatidine,administration significantly increased the peak power amplitude from 2.4 cpm or a lower frequency (bradygastria) to 3 cpm. Prior to nizatidine administration but after eating a meal, the peak power amplitude on EGG was not increased in Group DM without N. In Group DM with N, however, the EGG peak power amplitude increased to levels similar to those of the healthy subjects (Group C-II). Neither the single nor the chronic administration of nizatidine significantly prolonged the QT interval or increased the QT dispersion. A spectral analysis of

heart rate variability showed that nizatidine administration, whether acute or chronic, did not significantly change the indices of autonomic nervous activity. Nizatidine may promote gastric emptying by inhibiting acetylcholine esterase, thus increasing cholinergic activity, and by acting directly on gastric smooth muscle. The results indicate that because nizatidine increases gastric motility without exerting a negative influence on the autonomic nerves, it may be a useful drug in patients with diabetic neuropathy.

Chen *et al.*,[80] reported that nizatidine and omeprazole enhance the effect of metronidazole on Helicobacter pylori in vitro. Treatment failures are common in patients infected with metronidazole-resistant Helicobacter pylori in the gastric mucosa when triple therapy including metronidazole is used. In patients with treatment failure and metronidazole-resistant H. pylori, a higher eradication rate for H. pylori wasfound after secondary treatment with bismuth/ranitidine in combination with antibiotics including metronidazole, compared with the same antibiotics combined with a standard dose of omeprazole. This agrees with our previous finding that bismuth was able to reduce the susceptibility of H. pylori to metronidazole. In this study, we have found that nizatidine, an H(2)-receptor antagonist, is also able to reduce the susceptibility of H. pylori to metronidazole in vitro, despite having no direct inhibitory effect on the growth of H. pylori. This agrees with earlier findings that compounds having the ability to reverse antibiotic resistance do not necessarily have an antibiotic or chemotherapeutic effect in the sense of growth inhibition. Therefore, it was decided to investigate the effect of nizatidine and omeprazole on the oxidative respiratory chain, as it is known that metronidazole is able to inhibit the activity of fumarate reductase of H. pylori. This

enzyme is a key enzyme in the alternative respiratory chain under anaerobic conditions. Nizatidine was, in these preliminary experiments, found to inhibit fumarate reductase in a dose-dependent way, like metronidazole, whereas omeprazole had almost no effect on fumarate reductase. No other significant effects on the enzymes of the respiratory chain were found. The synergistic effect of nizatidine on metronidazole resistant H. pylori strains could be explained by the effect on fumarate reductase, whereas the effect of omeprazole is different and could be an inhibition of a proton pump in H. pylori. Reversal of antimicrobial resistance with the help of different non-antibiotics seems to be possible by using quite different compounds, and is therefore to be explained by different molecular mechanisms.

3. AIM AND OBJECTIVE

Drug repositioning (also known as drug repurposing or drug reprofiling) is commonly known as the process of redeveloping a compound for use in a different disease. Drug Repositioning usually has many benefits over traditional drug discovery approaches in that it can considerably reduce the cost and developmental time as many compounds have demonstrated safety in humans. Drug repositioning generally removes the need of phase 1 clinical trials.

Nizatidine has long been used as antiulcer drug and its safety in humans has been established. It has been reported to act through blocking H_2 Histamine receptor. The analgesic and anti-nociceptive activity of drugs belonging to this category such as ranitidine and famotidine has been reported earlier. The analgesic and anti-nociceptive activity of a drug candidate can be confirmed only in rodent models like mice and rats as they better mimic certain human metabolism, behavior and they are easy to handle.

Therefore based on the extensive literature survey, the objective of present work focused on probable anti-nociceptive and analgesic activity of nizatidine in rodent models.

The objectives are to evaluate the anti-nociceptive and analgesic activities of nizatidine in

a. Acetic acid induced writhing l
b. Hotplate test.
c. Tail immersion test

4. MATERIALS AND METHODS

Animals

Six weeks old male Swiss albino mice, weighing 20 ± 5 gms were used for this study. The animals were group housed (n=6 per cage) in a room with controlled temperature (21-22°C), and in normal light-dark cycle (12 h/12 h).They had free access to food and water *ad libitum.* All the experimental protocols employed in this study were approved by the Institutional Animal Ethical Committee of J.K.K. Nattraja College of Pharmacy and experiments were performed according to the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines on the ethical use of animals (JKKN/IAEC/M.Pharm/19/2019 dated 10/4/2019).

Drugs

Table 1. List of chemicals used in the present study

S. No.	Chemicals	Manufacturer
1.	Diclofenac	Cadila
2.	Nizatiine	Mylan

Drug solutions

Diclofenac was diluted with normal saline (0.9% NaCl) and administered at a dose of 10 mg/kg i.p. Nizatidine was suspended in normal saline and administered at a dose of 25 mg/kg p.o. and 50 mg/kg, p.o..

Table 2. Grouping of animals for evaluation of anti-nociceptive activity of Nizatidine in acetic aci induced wrthing hotplate and tail immersion tests

S.No	Group	Number of animals	Treatment
2	Group II	6	Standard (Diclofenac 10 mg/kg *Morphine 2mg/kg i.p.)
3	Group III	6	Nizatidine (25 mg/kg, p.o.)
4	Group IV	6	Nizatidine (50 mg/kg, p.o.)

Data obtained from research paper*

Acetic acid induced writhing[9]

Administration of chemical irritants such as acetic acid causes pain by releasing endogenous pain mediators. The mice were treated with standard drug or fractions, 60 min prior to the administration 0.7% acetic acid (10 ml/kg, i.p.). The mice were observed immediately after acetic acid administration and the number of writhing was counted for 30 min. Complete writhing considered when the animal showed contraction of the abdomen, elongation of the body, twisting of the trunk and/or pelvis ending with the extension of the limbs[45].

Hotplate test

Mice were placed on hotplate maintained at a temperature of 55± 1°C and basal reaction time of animal (forepaw licking, withdrawal of the paw(s) or jumping response) was recorded. The animals were treated with morphine or selected fractions and were placed on Eddy's hotplate maintained at a temperature of 55± 1°C. The reaction times were noted again at 30, 60, 90, and 120 min interval. A cutoff period of 20 s was set to avoid tissue damage in foot.

Tail immersion test

Mice were treated with standard or selected fractions and one to two cm of the tail was immersed in hot water kept at the temperature of 55± 1°C. Time latency to withdraw the tail was noted at 30, 60, 120 min after the treatment. To prevent the excessive tail tissue damage, cut off latency period of 20 s was maintained.

Acetic acid-induced writhing model

Administration of EAFCF (50, 100, and 200 mg/kg, p.o.) and NHFCA (100, 200 and 400 mg/kg, p.o.) reduced the acetic acid induced writhing significantly ($p<0.001$) compared to control group in dose dependent manner (table 21) and the reduction in writhing was observed as 91.07% in standard analgesic, diclofenac sodium (10 mg/kg, i.p.), treated animals and 40.18 %, 71.07% and 92.01% respectively, in 50,100 and 200 mg/kg of EAFCF treated animals.

Statistical analysis

The data obtained and expressed as mean ± SEM were evaluated by using ANOVA followed by Dunnet's post hoc.

5. RESULTS

Acetic acid-induced writhing model

Administration of nizatidine (25 and 50 mg/kg, p.o.) and reduced the acetic acid induced writhing significantly ($p<0.001$) compared to control group in dose dependent manner (table 3) and the reduction in writhing was observed as 91.07% in standard analgesic, diclofenac sodium (10 mg/kg, i.p.), treated animals and 40.18 %, 71.07% and 92.01% respectively, in 25 and 50 of nizatidine treated animals.

Table 3. Analgesic activity of nizatidine in acetic acid induced writhing in mice

Groups	Dose	Number of Writhing Mean± SEM
Vehicle	10 ml/kg, i.p.	68±1.390
Diclofenac	10 mg/kg, i.p.	7.83±0.477
Nizatidine	25 mg/kg, p.o.	41.33±1.520
Nizatidine	50 mg/kg, p.o	21.17±0.946*

Values are expressed in terms of mean ± SEM, n = 6 in each group, *P <0.01 statistically significant as compared with control group.

Eddy's hot plate model in mice

In this model, the reaction latency to thermal stimuli was increased significantly ($P<0.01$) in nizatidine treated groups compared to the control group. The maximum effect (reaction time of 18.4 s) was observed at the highest dose viz. 50 mg/kg p.o. at 60 min. while the standard drug morphine (1.5 mg/kg i.p.) showed highest reaction time of 17.8 s (Data obtained from reference article). The anti-nociceptive effect produced by nizatidine was found to be dose and time dependent (table 22).

Table 4. Anti-nociceptive effect of nizatidine in Eddy's hot plate model using mice

Group	Dose	Reaction time in sec				
		Before	**30 min**	**60 min**	**120 min**	**180 min**
Vehicle	10 ml/kg, i.p.	4.8±0.05	4.6±0.08	5.2±0.03	5.6±0.09	5.8±0.08
Morphine	1.5 mg/kg, i.p.	5.1±0.06	13.6±0.03*	18.8±0.08*	17.2±0.1*	15.8±0.2*
Nizatidine	25 mg/kg, p.o.	6.1±0.09	6.1±0.08*	8.4±0.08*	7.7±0.08*	6.2±0.08*
Nizatidine	50 mg/kg, p.o	5.2±0.10	10.8±0.05*	13.1±0.1*	12.5±0.3*	10.6±0.09*

Data expressed as mean ± SEM, n = 6 in each group, *P <0.01 statistically significant as compared with control group. i.p.=intra peritoneal, p.o.= per oral

Tail immersion test

The antinociceptive activity exhibited by nizatidine and morphine in tail immersion test is given in table 5. Nizatidine (25 & 50 mg/kg, p.o.) showed dose dependent increase in the reaction latency to hot-water induced thermal stimuli. ($p<0.01$). Morphine also produced similar effect as that of nizatidine 50mg/kg.

Table 5. Anti-nociceptive activity of nizatidine in mouse tail immersion test

Group	Dose	Reaction time in sec				
		Before	**30 min**	**60 min**	**120 min**	**180 min**
Vehicle	10 ml/kg, i.p.	4.8±0.05	4.6±0.01	5.43±0.04	5.7±0.09	5.58±0.08
Morphine	1.5 mg/kg, i.p.	4.9±0.07	13.6±0.03*	18.8±0.08*	18.2±0.1*	11.8±0.2*
Nizatidine	25 mg/kg, p.o.	5.5±0.1	6.1±0.08	9.4±0.08*	8.7±0.08*	9.2±0.08*
Nizatidine	50 mg/kg, p.o	5.9±0.20	10.8±0.05*	13.1±0.1*	12.5±0.3*	10.6±0.09*

Experimental data given as mean ± SEM, n = 6 in each group, *P <0.01 statistically significant as compared with control group.

6. DISCUSSION

Drug repositioning (also known as drug repurposing or drug reprofiling) is commonly known as the process of redeveloping a compound for use in a different disease. Drug Repositioning usually has many benefits over traditional drug discovery approaches in that it can considerably reduce the cost and developmental time as many compounds have demonstrated safety in humans. Drug repositioning generally removes the need of phase 1 clinical trials.

Nizatidine has long been used as antiulcer drug and its safety in humans has been established. It has been reported to act through blocking H_2 Histamine receptor. The analgesic and anti-nociceptive activity of drugs belonging to this category such as ranitidine and famotidine has been reported earlier. The analgesic and anti-nociceptive activities of a drug candidate can be confirmed only in rodent models like mice and rats as they better mimic certain human metabolism, behavior and they are easy to handle.

Pain is considered as a major symptom of various diseases including the CNS disorders that is capable to produce severe physical and psychological distress for many patients and the most predominant symptom affecting their quality of life. The widely accepted definition given for pain by International Association for the Study of Pain is "An unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage"[81]. Histamine H_2 receptor blockers such as ranitidine and famotidine used for the treatment of hyperacidity in ulcer condition have been reported to analgesic activity in acetic acid induced hyperaalgesia. However, the anti-nociceptive or analgesic

activity of nizadine, a most widely used antiulcer drug, has not been reported earlier. In this context, the present study was aimed in evaluation of nizatidine for antinociceptive and analgesic activities.

Nizatiine was evaluated for their nociceptive and analgesic activities in peripheral as well as central analgesic models. Acetic acid induced writhing in mice is simple and most reliable inflammatory pain model widely used for the evaluation of peripheral analgesics. The pain caused by acetic acid is said to be an inflammatory pain due to increase in the capillary permeability and release of endogenous mediators such as PGE1, PGE2, histamine, bradykinin, substance P etc… which sensitize the nociceptive nerve endings [129]. NSAIDs are known to inhibit the COX enzyme in the peripheral tissues which is responsible for the production of pain mediators. In this study, nizatiine showed dose dependent analgesic activity as evident through significant ($p<0.01$) reduction in number of writhing caused by acetic acid. Hence, nizatidine may act via blockade of the release or activity of endogenous pain mediators resulted in the interruption of pain stimuli transduction similar to that of the standard drug diclofenac sodium.

Treatment of nizatidine in mice, increased the reaction time significantly ($p<0.01$) to the thermal stimuli in both hotplate and tail immersion model. These two models are mainly used for centrally acting analgesics, while the peripheral analgesics are found to ineffective. The reaction to the hotplate demonstrates the supraspinal reflex and tail immersion explains the spinal reflex mediated by various sub-types of opioid receptors. Findings of the present study indicate that the nizatidine may act as an anti-nociceptive by central mechanisms.

This study also warrants further studies in the line of receptor binding assays and interaction with various neurochemical analogs which may be beneficial in exploring the molecular mechanism for the anti-nociceptive and analgesic activities of nizatidine.

7. SUMMARY AND CONCLUSION

Previous research reports revealed that histamine H_2 receptor blockers such as ranitidine and famotidine showed analgesic activity in rodent models. However, the analgesic or anti-nociceptive activity of nizatidine, a most widely used H_2 receptor blocker, has not yet been reported. Hence, the present study was aimed to evaluate the probable anti-nociceptive and analgesic activities of nizatidine in valid animal models. The results obtained in this study revealed the anti-nociceptive and analgesic activities of nizatidine. However, further chronic studies are required to validate the analgesic and anti-nociceptive activities of nizatidine. In conclusion, nizatiine produces the anti-nociceptive and analgesic activities in valid animal models similar to that of other H_2 receptor blockers.

8. REFERENCES

1. Merskey H, Albe-Fessard D, Bonica JJ, Carmon A, *et al*. Pain terms: a list with definitions and notes on usage. Recommended by the IASP Subcommittee on Taxonomy. Pain 1979; 6:249.

2. Cervero F, Laird JMA. One pain or many pains? A new look at pain mechanisms.News Physiol Sci 1991; 6:268–73

3. Fields HL, Basbaum AI. Central nervous system mechanisms of pain modulation. In: Wall PD, Melzack R (eds) Textbook of pain 1999. Churchill Livingstone, London ; 309–29

4. Bishop GH, Landau WM. Evidence for a Double Peripheral Pathway for Pain. Science. 1958;128(3326):712–713.

5. Sherman SM, Guillery R. Functional organization of thalamocortical relays. Journal of Neurophysiology. 1996; 76(3):1367.

6. Willis W, Westlund K. Neuroanatomy of the pain system and of the pathways that modulate pain. Journal of Clinical Neurophysiology. 1997; 14(1):2.

7. Tracey I, Mantyh PW. The cerebral signature for pain perception and its modulation. Neuron. 2007; 55(3):377–391.

8. Melzack R. From the gate to the neuromatrix. Pain. 1999; 82:S121–S126.

9. Petrenko AB, Yamakura T, Baba H, Shimoji K. The role of N-methyl-D-aspartate (NMDA) receptors in pain: a review. Anesthesia & Analgesia. 2003; 97(4):1108.

10. JM B. The neurobiology of pain. The Lancet. 1999; 353(9164):1610–1615.

11. Yaksh TL. Pharmacology of spinal adrenergic systems which modulate spinal nociceptive processing. Pharmacology Biochemistry and Behavior. 1985; 22(5):845–858.

12. Voisin DL, Guy N, Chalus M, Dallel R. Nociceptive stimulation activates locus coeruleus neurones projecting to the somatosensory thalamus in the rat. The Journal of Physiology. 2005; 566(3):929–937.

13. Yaksh TL. Opioid receptor systems and the endorphins: a review of their spinal organization. Journal of neurosurgery. 1987; 67(2):157–176.

14. Basbaum AI, Fields HL. Endogenous pain control systems: brainstem spinal pathways and endorphin circuitry. Annual Review of Neuroscience. 1984; 7(1):309–338.

15. Jasmin L, Rabkin SD, Granato A, et al. Analgesia and hyperalgesia from GABA-mediated modulation of the cerebral cortex. Nature. 2003; 424(6946):316–320.

16. Heinricher M, Tavares I, Leith J, Lumb B. Descending control of nociception: Specificity, recruitment and plasticity. Brain research reviews. 2009; 60(1):214–225.

17. Melzack R, Wall PD. Pain mechanisms: a new theory. Science. 1965; 150(699):971–979.

18. Reynolds DV. Surgery in the rat during electrical analgesia induced by focal brain stimulation. Science. 1969; 164(3878):444. [PubMed: 4887743]

19. Carlson JD, Maire JJ, Martenson ME, Heinricher MM. Sensitization of pain-modulating neurons in the rostral ventromedial medulla after peripheral nerve injury. The Journal of Neuroscience. 2007; 27(48):13222.

20. NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript20. Desimone R, Duncan J. Neural mechanisms of selective visual attention. Annu Rev Neurosci.1995; 18:193–222.

21. Corbetta M, Shulman GL. Control of goal-directed and stimulus-driven attention in the brain.Nature Reviews: Neuroscience. 2002; 3:201–215.

22. Friedman RS, F\örster J. Implicit affective cues and attentional tuning: An integrative review. Psychological bulletin. 2010; 136(5):875.

23. Lang PJ, Bradley MM. Emotion and the motivational brain. Biol Psychol. 2011; 84:437–50.

24. Legrain V, Perchet C, García-Larrea L. Involuntary orienting of attention to nociceptive events: neural and behavioral signatures. Journal of neurophysiology. 2009; 102(4):2423.

25. Eccleston C, Crombez G. Pain demands attention: A cognitive–affective model of the interruptive function of pain. Psychological Bulletin. 1999; 125(3):356.

26. Quevedo AS, Coghill RC. Attentional modulation of spatial integration of pain: evidence for dynamic spatial tuning. The Journal of Neuroscience. 2007; 27(43):11635–11640.

27. Terkelsen AJ, Andersen OK, MU00F8lgaard H, Hansen J, Jensen T. Mental stress inhibits pain perception and heart rate variability but not a nociceptive withdrawal reflex. Acta physiologica scandinavica. 2004; 180(4):405–414.

28. Wiech K, Ploner M, Tracey I. Neurocognitive aspects of pain perception. Trends in Cognitive Sciences. 2008; 12(8):306–313.

29. Schoth DE, Nunes VD, Liossi C. Attentional bias towards pain-related information in chronic pain; a meta-analysis of visual-probe investigations. Clinical Psychology Review. 2011

30. Hollins M, Harper D, Gallagher S, et al. Perceived intensity and unpleasantness of cutaneous and auditory stimuli: An evaluation of the generalized hypervigilance hypothesis. PAIN. 2009; 141(3): 215–221.

31. Rollman GB. Perspectives on hypervigilance. PAIN. 2009; 141(3):183–184.

32. Rainville P, Carrier B, Hofbauer RK, Bushnell MC, Duncan GH. Dissociation of sensory and affective dimensions of pain using hypnotic modulation. Pain. 1999; 82:159–71.

33. Rainville P, Duncan GH, Price DD, Carrier B, Bushnell MC. Pain affect encoded in human anterior cingulate but not somatosensory cortex. Science. 1997; 277:968–71.

34. Price DD. Central neural mechanisms that interrelate sensory and affective dimensions of pain. Mol Interv. 2002; 2(6):392–403.

35. 35. Ochsner KN, Gross JJ. The cognitive control of emotion. Trends in Cognitive Science. 2005; 9:242–9.

36. Kalisch R. The functional neuroanatomy of reappraisal: time matters. Neurosci Biobehav Rev. 2009; 33:1215–26.

37. Haythornthwaite JA, Menefee LA, Heinberg LJ, Clark MR. Pain coping strategies predict perceived control over pain. Pain. 1998; 77(1):33–39.

38. Garland EL, Gaylord SA, Palsson O, et al. Therapeutic mechanisms of a mindfulness-based treatment for IBS: effects on visceral sensitivity, catastrophizing, and affective processing of pain sensations. Journal of Behavioral Medicine. 2011:1–12.

39. Severeijns R, Vlaeyen JWS, van den Hout MA, Weber WEJ. Pain catastrophizing predicts pain intensity, disability, and psychological distress independent of the level of physical impairment. The Clinical journal of pain. 2001; 17(2):165.

40. Picavet HSJ, Vlaeyen JWS, Schouten JSAG. Pain Catastrophizing and Kinesiophobia: Predictors of Chronic Low Back Pain. American Journal of Epidemiology. 2002; 156(11):1028–1034.

41. Tousignant-Laflamme Y, Marchand S. Sex differences in cardiac and autonomic response to clinical and experimental pain in LBP patients. European Journal of Pain. 2006; 10(7):603–614.

42. Flor H, Turk DC, Birbaumer N. Assessment of stress-related psychophysiological reactions in chronic back pain patients. Journal of Consulting and Clinical Psychology. 1985; 53(3):354–364. Journal of Consulting and Clinical Psychology.

43. Lundberg U, Dohns IE, Melin B, et al. Psychophysiological stress responses, muscle tension, and neck and shoulder pain among supermarket cashiers. Journal of Occupational Health Psychology. 1999; 4(3):245–255. Journal of Occupational Health Psychology.

44. Rainville P, Bao QVH, Chrétien P. Pain-related emotions modulate experimental pain perception and autonomic responses. Pain. 2005; 118(3):306–318.

45. Cannon WB. Organization of physiological homeostasis. Physiology Review. 1929; 946.

46. Benarroch EE. Pain-autonomic interactions. Neurological Sciences. 2006; 27(S2):s130–s133.

47. Sommer C, Kress M. Recent findings on how proinflammatory cytokines cause pain: peripheral mechanisms in inflammatory and neuropathic hyperalgesia. Neuroscience Letters. 2004; 361(1–3): 184–187.

48. Chapman CR, Tuckett RP, Song CW. Pain and stress in a systems perspective: reciprocal neural, endocrine, and immune interactions. The Journal of Pain. 2008; 9(2):122–145.

49. Craig AD. Interoception: the sense of the physiological condition of the body. Curr Opin Neurobiol. 2003; 13:500–5.

50. Wiech K, Tracey I. The influence of negative emotions on pain: behavioral effects and neural mechanisms. Neuroimage. 2009; 47:987–94.

51. de Wied M, Verbaten MN. Affective pictures processing, attention, and pain tolerance. Pain. 2001; 90(1-2):163–172.

52. Kirwilliam SS, Derbyshire SWG. Increased bias to report heat or pain following emotional priming of pain-related fear. PAIN. 2008; 137(1):60–65.

53. Bogaerts K, Janssens T, De Peuter S, Van Diest I, Van den Bergh O. Negative affective pictures can elicit physical symptoms in high habitual symptom reporters. Psychology & Health. 2009; 25(6):685–698.

54. Panerai AE. Pain emotion and homeostasis. Neurological Sciences. 2011; 32(S1):27–29.

55. Strigo IA, Simmons AN, Matthews SC, Craig AD (Bud), Paulus MP. Increased Affective Bias Revealed Using Experimental Graded Heat Stimuli in Young Depressed Adults: Evidence of “Emotional Allodynia.”. Psychosomatic Medicine. 2008; 70(3):338–344.

56. Keogh E, Ellery D, Hunt C, Hannent I. Selective attentional bias for pain-related stimuli amongst pain fearful individuals. Pain. 2001; 91:91–100.

57. Arnsten AFT. Stress signalling pathways that impair prefrontal cortex structure and function.Nature Reviews Neuroscience. 2009; 10(6):410–422.

58. Lawrence JM, Hoeft F, Sheau KE, Mackey SC. Strategy-dependent Dissociation of the Neural Correlates Involved in Pain Modulation. Anesthesiology. 2011; 115(4):844–851.

59. Keefe FJ, Wilkins RH, Cook WA. Direct observation of pain behavior in low back pain patients during physical examination. Pain. 1984; 20(1):59–68.

60. Hadjistavropoulos T, Craig KD, Fuchs-Lacelle S. Social influences and the communication of pain. Pain: psychological perspectives. 2004:87–112.

61. Turk DC, Flor H. Pain> pain behaviors: the utility and limitations of the pain behavior construct. Pain. 1987; 31(3):277–295.

62. Vlaeyen JWS, Linton SJ. Fear-avoidance and its consequences in chronic musculoskeletal pain: a state of the art. Pain. 2000; 85(3):317–332.

63. Linton SJ, Buer N, Vlaeyen J, Hellsing AL. Are fear-avoidance beliefs related to the inception of an episode of back pain? A prospective study. Psychology and Health. 2000; 14(6):1051–1059.

64. Buer N, Linton SJ. Fear-avoidance beliefs and catastrophizing: occurrence and risk factor in back pain and ADL in the general population. Pain. 2002; 99(3):485–491. 9

65. Klenerman L, Slade P, Stanley I, et al. The prediction of chronicity in patients with an acute attack of low back pain in a general practice setting. Spine. 1995; 20(4):478.

66. Crombez G, Vlaeyen JWS, Heuts PHTG, Lysens R. Pain-related fear is more disabling than pain itself: evidence on the role of pain-related fear in chronic back pain disability. Pain. 1999; 80(1-2): 329–339.

67. Waddell G, Newton M, Henderson I, Somerville D, Main CJ. A Fear-Avoidance Beliefs Questionnaire (FABQ) and the role of fear-avoidance beliefs in chronic low back pain and disability. Pain. 1993; 52(2):157–168.

68. Vlaeyen JWS, Kole-Snijders AMJ, Boeren RGB, Van Eek H. Fear of movement/(re) injury in chronic low back pain and its relation to behavioral performance. Pain. 1995; 62(3):363–372.

69. Netti C, Guidobono F, Sibilia V, Villa I, Cazzamalli E, Pecile A. Central effects of histamine H2-receptor agonists and antagonists on nociception in the rat. Agents Actions. 1988 Apr;23(3-4):247-9

70. Ahmadi A(1), Ebrahimzadeh MA, Ahmad-Ashrafi S, Karami M, Mahdavi MR, Saravi SS. Hepatoprotective, antinociceptive and antioxidant activities of cimetidine, ranitidine and famotidine as histamine H2 receptor antagonists. Fundam Clin Pharmacol. 2011 Feb;25(1):72-9.

71. Bethesda Histamine Type-2 Receptor Antagonists (H2 Blockers). LiverTox: Clinical and Research Information on Drug-Induced Liver Injury : National Institute of Diabetes and Digestive and Kidney Diseases; 2012-.2018 Jan 25.

72. Sanad MH, Saleh GM, Marzook FA. Radioiodination and biological evaluation of nizatidine as a new highly selective radiotracer for peptic ulcer disorder detection. J Labelled Comp radiopharm. 2017 Nov;60(13):600-607.

73. Yamaji Y(1), Omata T, Abe T, Yoshida A, Ueki S, Aita H, Morita H, Chaki K, Segawa Y, Kurimoto T, et al. Effects of successive doses of nizatidine, cimetidine and ranitidine on serum gastrin level and gastric acid secretion. Arzneimittelforschung. 1991 sep;41(9):954-7.

74. Probst KS, Higdon GL, Fisher LF, McGrath JP, Adams ER, Emmerson JL. Preclinical toxicology studies with nizatidine, a new H2-receptor antagonist: acute, subchronic, and chronic toxicity evaluations. Fundam Appl Toxicol. 1989 Nov;13(4):778-92.

75. Kim JY, Kim KS, Kim IS, Yoon S. Histamine Receptor Antagonists, Loratadine and Azelastine, Sensitize P-gp-overexpressing Antimitotic Drug-resistant KBV20C Cells Through Different Molecular Mechanisms. Anticancer Res. 2019 Jul;39(7):3767-3775

76. Morrissey KM, Stocker SL, Chen EC, Castro RA, Brett CM, Giacomini KM. The Effect of Nizatidine, a MATE2K Selective Inhibitor, on the Pharmacokinetics and pharmacodynamics of Metformin in Healthy Volunteers. Clin Pharmacokinet. 2016 Apr;55(4):495-506.

77. Liu J(1), Sun D(1), He J(1), Yang C(1), Hu T(1), Zhang L(1), Cao H(1), Tong AP(1), Song X(1), Xie Y(1), He G(1), Guo G(1), Luo Y(1), Cheng P(2), Zheng. Gastroprotective effects of several H2RAs on ibuprofen-induced gastric ulcer in rats.Life Sci. 2016 Mar 15;149:65-71.

78. Dahan A(1), Sabit H, Amidon GL. The H2 receptor antagonist nizatidine is a P-glycoprotein substrate: characterization of its intestinal epithelial cell efflux transport. AAPS J. 2009 Jun;11(2):205-13.

79. Tomokane Y(1), Nomura M, Kujime S, Noda Y, Kondo N, Nakaya Y, Ito S. Clinical study on the effects of nizatidine on gastric motility and cardiac autonomic function. Investigations using electrogastrography and spectral analysis of heart rate variability. Arzneimittelforschung. 2004;54(8):427-35.

80. Chen M, Jensen B, Zhai L, Colding H, Kharazmi A, Kristiansen JE, Andersen LP. Nizatidine and omeprazole enhance the effect of metronidazole on Helicobacter pylori in vitro. Int J Antimicrob Agents. 2002 Mar;19(3):195-200.

CERTIFICATE

Name of the student : **M. Kanagaraj**

This is to certify that the project "Evaluation Of Anti-Nociceptive And Analgesic Activities Of Nizatidine In Mice" has been approved by the IAEC, meeting held on 10-04-2019.

Proposal number : **JKKN/IAEC/M.Pharm/19/ 2019**

Approval date : **10-04-2019**

No of animals sanctioned : **12 Mice**

10/4/19

Dr R.Sambath kumar

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