UNIVERSITI PUTRA MALAYSIA

ANTI-ALLODYNIC AND ANTIHYPERALGESIC EFFECTS OF ZERUMBONE THROUGH INVOLVEMENT OF MONOAMINERGIC PATHWAYS IN MICE MODEL OF NEUROPATHIC PAIN

JASMINE CHIA SIEW MIN

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By

JASMINE CHIA SIEW MIN

Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

June 2018
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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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JASMINE CHIA SIEW MIN

June 2018

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Faculty : Medicine and Health Sciences

Neuropathic pain is a pain condition that arises following an injury to the nervous system, persisting past the presence of any noxious stimulus or inflammation. The complex and multifaceted mechanisms underlying the development of neuropathic pain is yet to be fully understood. For this reason, treatments available for neuropathic pain patients are not specific and do not provide sufficient pain relief. Zerumbone is the major bioactive compound found in rhizomes of the Zingiber zerumbet (L.) Smith ginger plant. Zerumbone has shown to possess multiple pharmacological potentials, most importantly in exhibiting anti-allodynic and antihyperalgesic effects in a neuropathic pain animal model. Therefore, the present study was conducted to explore the mechanisms mainly the inhibitory monoaminergic system, in the antineuropathic properties of zerumbone in a chronic constriction injury (CCI)-induced neuropathic pain mice model. The chronic constriction injury model on the sciatic nerve was employed in ICR male mice to develop neuropathic pain. The determination of the effective dosage of zerumbone in exhibiting its anti-allodynic and antihyperalgesic effects were conducted by intraperitoneal administration of zerumbone at 5, 10 and 50 mg kg on Day 14 following CCI surgery. Behavioural responses were assessed using the von Frey filament test (mechanical allodynia) and Hargreaves plantar test (thermal hyperalgesia). Zerumbone at 10 mg kg exhibited significant anti-allodynic and antihyperalgesic effects in the CCI neuropathic pain mice. The monoaminergic system is mainly controlled by serotonergic and noradrenergic projections and receptors. Investigation into the involvement of the serotonergic system was conducted by firstly depleting serotonin levels using p-chlorophenylalanine (PCPA), whereby zerumbone’s antineuropathic properties were significantly reversed in both von Frey filament and Hargreaves plantar tests. Following this, pre-administration of specific 5-HT receptor subtype antagonists prior to zerumbone (10 mg kg) treatment significantly indicated the involvement of 5-HT
receptor subtypes 1A, 1B, 2A, 3, 6 and 7. Further investigation revealed an increase in 5-HT1A receptor expression in mice brain samples following zerumbone treatment in CCI-induced neuropathic pain mice, analysed using Western Blot. Following this, the involvement of the noradrenergic system was initially investigated by administering non-specific α- and β-adrenergic receptor antagonists prior to zerumbone. Further investigation into specific adrenergic receptor subtypes revealed that mainly α1, α2- and β2-adrenergic receptors are necessary for zerumbone to exhibit its antineuropathic properties. The decrease in α2A-adrenergic receptor expression suggests the inhibitory action of zerumbone against the upregulation of these receptors following nerve injury, which has shown to facilitate nociceptive transmission. To look further into the mechanisms of action of zerumbone, prominent receptors and systems involved in the pain pathway that are known to enhance the inhibitory tone of the monoaminergic system were elucidated. The findings demonstrated the involvement of TRPV, NMDA, cannabinoid CB1 and PPARδ, PPARγ receptors in zerumbone exhibiting its antiallodynic and antihyperalgesic effects in neuropathic pain conditions. Moreover, expression of TRPV1 and NMDA NR2B receptors increased following zerumbone treatment. Data were analysed using One-way Analysis of Variance (ANOVA) followed by Tukey’s post hoc test, with the level of significance set at p < 0.05. In conclusion, the current study elucidated the involvement of the monoaminergic □ serotonergic and noradrenergic, vanilloid, glutamatergic, cannabinoid and nuclear hormone receptor systems in zerumbone’s antineuropathic properties.
Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

SIFAT ANTI-ALLODINIA DAN ANTIHIPERALGESIA ZERUMBONE MENERUSI PENGLIBATAN DALAM MONOAMINERGIK DALAM MODEL MENCIT KESAKITAN NEUROPATIK

Oleh

JASMINE CHIA SIEW MIN

Jun 2018

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Kesakitan neuropatik adalah kondisi kesakitan yang disebabkan oleh kecederaan pada sistem saraf yang berterusan melangkah kehadiran sebarang rangsangan berbahaya atau keradangan. Mekanisme kompleks dan berbagai fungsi yang menjadi asas pembentukan kesakitan neuropatik belum lagi difahami sepenuhnya. Untuk sebab ini, rawatan sedia ada untuk pesakit neuropatik tidak khusus dan tidak memberi keleluasaan kesakitan yang mencukupi. Zerumbone adalah sebatian bioaktif utama yang terdapat dalam rizom tumbuhan halia Zingiber zerumbet (L.) Smith. Zerumbone mempunyai pelbagai potensi farmakologi, terutamanya dalam menunjukkan kesan anti-allodinia dan antihiperalgesia dalam model haiwan kesakitan neuropatik. Oleh itu, kajian ini dijalankan untuk menyelidik mekanisme yang terlibat, terutamanya sistem penginhibitan monoaminergik, dalam sifat antineuropatik zerumbone pada tikus neuropatik diinduksi oleh model haiwan kecederaan konstraksi kronik. Kecederaan konstraksi kronik (CCI) pada saraf siatik digunakan pada tikus jantan ICR untuk pembentukan kesakitan neuropatik. Penentuan dos berkesan zerumbone dalam mempamerkan kesan anti-allodinia dan antihiperalgesia telah dijalankan dengan suntikan intraperitoneum zerumbone pada 5, 10 dan 50 mg/kg pada Hari ke-14 selepas pembedahan CCI. Tindak balas tingkah laku telah dinilai menggunakan ujian filamen von Frey (allodinia mekanikal) dan ujian planlar Hargreaves. Zerumbone pada 10 mg mempamerkan kesan anti-alloidinia and antihiperalgesia signifikan pada tikus CCI neuropatik. Sistem monoaminergik diwakal terutamanya oleh proyeksi dan reseptor serotonergik dan noradrenergik. Siastan ke atas penglibatan sistem serotonergik telah dilakukan dengan mengurangkan tahap serotonin menggunakan γ-chlorophenylalanine (PCPA), dimana sifat neuropatik zerumbone dibalikkan dengan signifikan dalam kedua-dua ujian filamen von Frey dan planlar Hargreaves. Berikut ini, pemberian antagonis subjenis reseptor 5-HT sebelum rawatan zerumbone (10 mg/kg) menunjukkan penglibatan subjenis reseptor 5-HT 1A, 1B,
2A, 3, 6 dan 7 yang signifikan. Siasatan lanjut mendedahkan peningkatan dalam ekspresi reseptor 5-HT$_{1A}$ pada sampel otak mencit berikut rawatan zerumbone dalam tisu CCI neuropatik, dianalisis dengan menggunakan Western Blot. Berikut ini, penglibatan sistem noradrenergik pada mulanya disiasat dengan memberi antagonis tidak khusus reseptor $\alpha$- dan $\beta$-adrenergik sebelum rawatan zerumbone. Siasatan lanjut pada subjensi reseptor adrenergik spesifik mendedahkan bahawa reseptor $\alpha_1$, $\alpha_2$- dan $\beta$-adrenergik diperlukan untuk zerumbone mempamerkan sifat antineuropatiknya. Pengurangan expresi reseptor $\alpha_2A$-adrenergik mencadangkan bahawa fndakan penginhibitan zerumbone terhadap pengawalaturan meningkat reseptor ini berikut kecederaan saraf, yang telah ditunjukkan untuk memudahkan pengaliran nosiseptif. Untuk menyiasat dengan lebih mendalam lagi tentang mekanisme fndakan zerumbone, reseptor dan sistem yang terlibat dalam laluan kesakitan yang diketahui untuk meningkatkan pada penginhibitan sistem monoaminergik telah dijelaskan. Penemuan kajian menunjukkan penglibatan zerumbone dengan reseptor TRPV, NMDA, cannabinoid CB$_1$ dan PPAR$_{\alpha}$, PPAR$_{\gamma}$ dalam mempamerkan kesan anti-allodinik dan antihiperalgesik dalam kondisi sakit neuropatik. Tambahan lagi, ekspresi reseptor TRPV1 dan NMDA NR2B meningkat berikut rawatan zerumbone. Data dianalisis menggunakan analisis varians (ANOVA) sehala diikuti oleh ujian post hoc Tukey, dengan tahap keertian statistik ditetapkan pada p 0.05. Kesimpulannya, kajian semasa menjelaskan penglibatan sistem monoaminergik, serotonergik dan noradrenergik, vanilloid, glutamatergik, cannabinoid dan reseptor hormon nuklear dalam sifat antineuropatik zerumbone.
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I am also deeply grateful to all the staffs and colleagues of the Physiology and Cell Signalling Laboratories in providing all the necessary support for this project.
I certify that a Thesis Examination Committee has met on 28 June 2018 to conduct the final examination of Jasmine Chia Siew Min on her thesis entitled "Anti-Allodynic and Antihyperalgesic Effects of Zerumbone Through Involvement of Monoaminergic Pathways in Mice Model of Neuropathic Pain" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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8.4 The effect of PPAR antagonists co-administration with vehicle on thermal hyperalgesia in CCI-induced neuropathic pain mice
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<td>5-HT</td>
<td>Serotonin</td>
</tr>
<tr>
<td>AC</td>
<td>Adenyl Cyclase</td>
</tr>
<tr>
<td>ACC</td>
<td>Anterior Cingulate Cortex</td>
</tr>
<tr>
<td>AMPA</td>
<td>α-amino-3-hydroxy-5-methyl-4-isoxazopropionic Acid</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
</tr>
<tr>
<td>AP-1</td>
<td>Activator Protein-1</td>
</tr>
<tr>
<td>AR</td>
<td>Adrenergic Receptors</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine Triphosphate</td>
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<tr>
<td>BDNF</td>
<td>Brain-derived Neurotrophic Factor</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>Calcium Ion</td>
</tr>
<tr>
<td>CB</td>
<td>Cannabinoid</td>
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<tr>
<td>CCI</td>
<td>Chronic Constriction Injury</td>
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<tr>
<td>CGRP</td>
<td>Calcitonin Gene Related Peptide</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>Chloride Ion</td>
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<tr>
<td>CNS</td>
<td>Central Nervous System</td>
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<tr>
<td>COX</td>
<td>Cyclooxygenase</td>
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<tr>
<td>DAG</td>
<td>Diacyl Glycerol</td>
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<tr>
<td>DMSO</td>
<td>Dimethyl Sulfoxide</td>
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<tr>
<td>DNIC</td>
<td>Diffuse Noxious Inhibitory Control</td>
</tr>
<tr>
<td>DRG</td>
<td>Dorsal Root Ganglion</td>
</tr>
<tr>
<td>DRN</td>
<td>Dorsal Raphe Nucleus</td>
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<tr>
<td>GABA</td>
<td>γ-aminobutyric acid</td>
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<tr>
<td>i.p.</td>
<td>Intraperitoneal</td>
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<tr>
<td>IASP</td>
<td>International Association for the Study of Pain</td>
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<tr>
<td>ICR</td>
<td>Imprinting Control Region</td>
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<tr>
<td>IL</td>
<td>Interleukin</td>
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<tr>
<td>IP₃</td>
<td>Inositol Triphosphate</td>
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<tr>
<td>K⁺</td>
<td>Potassium Ion</td>
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<tr>
<td>KA</td>
<td>Kainic Acid</td>
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<tr>
<td>LC</td>
<td>Locus Coeruleus</td>
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<td>Mg²⁺</td>
<td>Magnesium Ion</td>
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<tr>
<td>NA</td>
<td>Noradrenaline</td>
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<tr>
<td>Na⁺</td>
<td>Sodium Ion</td>
</tr>
<tr>
<td>NaCl</td>
<td>Sodium Chloride (Normal Saline)</td>
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<td>NF-κB</td>
<td>Nuclear Factor-κB</td>
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<tr>
<td>NGF</td>
<td>Nerve Growth Factor</td>
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<tr>
<td>NMDA</td>
<td>N-methyl-D-aspartate</td>
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<tr>
<td>NO</td>
<td>Nitric Oxide</td>
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<tr>
<td>NOS</td>
<td>Nitric Oxide Synthase</td>
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<td>PAG</td>
<td>Periaqueductal Gray</td>
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<td>PCPA</td>
<td>p-chlorophenylalanine</td>
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<td>PLC</td>
<td>Phospholipase C</td>
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<td>PPAR</td>
<td>Peroxisome Proliferator-activated Receptor</td>
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<td>PVDF</td>
<td>Polyvinylidene Fluoride</td>
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<td>RVN</td>
<td>Rostral Ventromedial Medulla</td>
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<tr>
<td>SNRI</td>
<td>Serotonin-Noradrenaline Reuptake Inhibitor</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
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<tr>
<td>STAT</td>
<td>Signal Transducer and Activator of Transcription</td>
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<tr>
<td>TBS</td>
<td>Tris-buffered Saline</td>
</tr>
<tr>
<td>TBST</td>
<td>Tris-buffered Saline with Tween 20</td>
</tr>
<tr>
<td>TCA</td>
<td>Tricyclic Antidepressants</td>
</tr>
<tr>
<td>TNF</td>
<td>Tumor Necrosis Factor</td>
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<tr>
<td>TRP</td>
<td>Transient Receptor Potential</td>
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<td>WDR</td>
<td>Wide Dynamic Range</td>
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CHAPTER 1

INTRODUCTION

Pain is a common unpleasant sensation felt by every individual. Acute pain serves only as a warning sign to avert our body from any harmful stimuli. In contrast to acute pain, neuropathic pain persists although the harmful stimuli are no longer present. Neuropathic pain, defined as a lesion to the somatosensory nervous system, is becoming a major health issue (Dworkin, 2002). This chronic pain condition could be caused either through direct lesion, compression or injury to nerves from trauma, infections, metabolic diseases or drugs. Common diseases that are at high risk in developing neuropathic pain are diabetes, postherpetic neuralgia and multiple sclerosis (Sadosky et al., 2008; Smith and Chong, 2000).

Neuropathic pain greatly impacts a patient’s life, affecting their daily routine. The common sensations experienced are burning sensations, stabbing or shooting pain that either arises spontaneously, intermittently or only when evoked. These sensations that arises significantly affects the patient’s mood, lifestyle and activities (Deshpande et al., 2006; Gilron et al., 2006; Vinik et al., 2005). The prevalence of neuropathic pain was reported to be in the range of 1-3% (Bouhassira et al., 2008; Bowsher, 1991; Dworkin et al., 2003; Irving, 2005). Based on reports by Cardosa et al. (2008) about 7% of 30,000 Malaysian adults suffer from neuropathic pain. The development of this chronic pain condition is complex and does not have any singularity in its cause. As the population continues to age, the prevalence of neuropathic pain is expected to increase (Galluzzi, 2005).

In comparison to neuropathic pain, acute pain patients are treated based on the site of injury. Neuropathic pain patients on the other hand would have to go through multiple hospital visits to screen, assess and diagnose patients (Kerstman et al., 2013; Ro and Chang, 2005). Common symptoms that arise due to neuropathic pain are hyperalgesia, allodynia, paroxysms and hyperpathia (Woolf and Mannion, 1999). Medications for neuropathic pain patients are prescribed after thorough assessment of the patient’s health conditions with referment to pharmacological guidelines. The current first line of treatment is tricyclic antidepressants (Finnerup et al., 2005). Based on the pharmacological guidelines for neuropathic pain patients, a combination of medications is often prescribed to ensure efficient analgesic effects, as compared to a monotherapy strategy (Gilron et al., 2005; Gilron and Max, 2005).

Unfortunately, many neuropathic pain patients do not receive sufficient pain relief due to the challenging pathophysiology behind neuropathic pain development, inaccurate medical treatments and comorbid conditions of patients. The two main key problems with current treatments for neuropathic pain are the insufficient pain
relief and side effects that often accompany these medications (T’lle, 2010). With an estimated increase in neuropathic pain incidence worldwide due to the aging population, there is a need for a novel compound that will specifically treat neuropathic pain.

Many invaluable studies have been conducted to further explore the mechanisms behind the development of neuropathic pain and the endogenous system modulating nociceptive transmission. Multiple underlying mechanisms and pathways have been discovered (Campbell and Meyer, 2006; Perl, 2011). The monoaminergic system, which is controlled by serotonergic and noradrenergic tones, acts synergistically in inhibiting nociceptive transmission. Additionally, the inhibitory action against nociceptive transmission is a summative effect from influences of afferent pathways — vanilloid, glutamate, cannabinoid, and nuclear hormone receptor systems (Millan, 2002).

Over the past three decades, many researches have been conducted in new drug discovery and development involving natural products (Newman and Cragg, 2012, 2016). Due to the incompatibility and high probability of causing severe side effects, synthetic compounds are often avoided. Asian countries, mainly India and China, are known to have the widest and richest array of medicinal plants (Kala et al., 2006; Tuintans et al., 2014). Many now-known drugs have been either derived or are particularly similar to compounds found in natural products. In addition, natural products have been used as folkloric medicine for decades to treat various illnesses.

Ginger plants have garnered extreme attention due to its anti-inflammatory and anticancer properties, typically in South-East Asia. The rhizomes of ginger plants are known for its medicinal properties, more than its pinecone counterpart. The rhizomes have been traditionally used to treat various illnesses of inflammation and pain — fever, constipation, stomachache (Elliott and Brimacombe, 1987; Norulaini et al., 2009; Perry and Metzger, 1980; Sulaiman et al., 2010b). Zingiber zerumbet (L.) Smith, of the Zingiber genus is a ginger plant known for its rich medicinal values. Z. zerumbet is widely distributed in Southeast Asia and the Pacific Islands (Burkill, 1966; Jantan et al., 2003; Larsen et al., 1999).

Zerumbone, the major active component of Z. zerumbet rhizomes, has been widely studied to assess its pharmacological properties (for review on previous studies, see (Yob et al., 2011)). Most importantly, zerumbone has shown prominent analgesic properties in acute and chronic pain animal models (Sulaiman et al., 2009; Zulazmi et al., 2015). Furthermore, zerumbone has displayed no toxicity at low to moderate dosages in acute toxicity studies, indicating safety of oral consumption (Ibrahim et al., 2010; Rahman et al., 2014; Sulaiman et al., 2010a).
Based on current available literatures, the opioidergic, serotonergic, cannabinoid, NMDA and TRPV systems are highly targeted by natural products to exhibit potent analgesic activity in neuropathic pain conditions (Kissin, 2010; Quintans et al., 2014). Furthermore, the underlying mechanisms behind the production of neuropathic pain and the mechanisms involved in active compounds must be clearly understood to allow synthesis of a more potent and specific treatment of targeted diseases.

Therefore, zerumbone is hypothesized to attenuate neuropathic pain symptoms: allodynia and hyperalgesia, by acting through serotonergic, noradrenergic, vanilloid, glutamatergic, cannabinoid and peroxisome proliferator-activated systems. The findings of this study will contribute important insights to advance our knowledge on zerumbone and its significance in treating neuropathic pain.

Objectives of the Study

The objectives of this study are to:

1. determine the effective dosage of zerumbone in attenuating allodynia and hyperalgesia in CCI-induced neuropathic pain mice;
2. investigate the involvement of serotonergic receptor subtypes and changes in 5-HT_{1A} receptor expression in the anti-allodynic and antihyperalgesic properties of zerumbone in CCI-induced neuropathic pain mice;
3. investigate the involvement of noradrenergic receptor subtypes and changes in \(\alpha_2\) adrenergic receptor expression in the anti-allodynic and antihyperalgesic properties of zerumbone in CCI-induced neuropathic pain mice;
4. investigate the involvement of TRPV and NMDA receptors and changes in TRPV VR1 and NMDA NR2B receptors expression in the anti-allodynic and antihyperalgesic properties of zerumbone in CCI-induced neuropathic pain mice;
5. investigate the involvement of cannabinoid receptors in the anti-allodynic and antihyperalgesic properties of zerumbone in CCI-induced neuropathic pain mice;
6. investigate the involvement of peroxisome proliferator-activated receptors in the anti-allodynic and antihyperalgesic properties of zerumbone in CCI-induced neuropathic pain mice.
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