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Original article

# Neuroprotective effect of liquiritin against neuropathic pain induced by chronic constriction injury of the sciatic nerve in mice

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ABSTRACT

Managing of neuropathic pain remains clinically challenging because the existing pharmacotherapies are either ineffective or non-specific. Therefore, developing novel alternatives is essential for better treatment. Liquiritin is an active component extracted from *Glycyrrhizae* radix and has potential neuroprotective action. This study aimed to investigate the protective efficacy of liquiritin on chronic constriction injury (CCI)-induced neuropathic pain in mice. Liquiritin (30, 60, and 120 mg/kg) and pregabalin (40 mg/kg) were administered intragastrically for 7 consecutive days starting on the 8th day post-surgery. Behavioral parameters and sciatic functional index were assessed on days 0, 7, 8, 10, 12, and 14. Electrophysiological and histopathological changes were analyzed on the 14th day. Immunofluorescence and Western blot were used to evaluate the expression of glial cells and the protein levels of inflammatory cytokines in the spinal cord, respectively. Results showed that liquiritin dose-dependently reduced hyperalgesia and allodynia and increased the sciatic functional index and motor nerve conduction velocities. Moreover, liquiritin restored the injured axon and myelin sheath, inhibited the activation of astrocyte and microglia, down-regulated the pro-inflammatory cytokines including tumor necrosis factor alpha (TNF-α), interleukin (IL-6, and IL-1β), and simultaneously up-regulated the anti-inflammatory cytokine IL-10. Our study revealed that liquiritin exerted a neuroprotective effect on CCI-induced neuropathic pain, which might be attributed to its direct protective effect on damaged nerves and its anti-inflammatory activity at the level of the spinal cord. Therefore, liquiritin shows promise as a compound for the development of novel analgesic agents that can be used to effectively treat intractable neuropathic pain.



## 1. Introduction

Neuropathic pain (NP) is a major chronic pain caused by aberrant sensory processing in peripheral and/or central nervous system (CNS) and is commonly associated with autoimmune disorders, metabolic diseases, infection, cancer, and traumatic nerve injury [1]. The total prevalence of chronic pain with neuropathic peculiarities is estimated to be 20.3% [2], whereas neuropathic pain influences about 18% of the general population suffering and bearing a financial burden to their families and the society in America [3]. The International Association for the Study of Pain (IASP) defined neuropathic pain as "pain caused by a lesion or disease of the somatosensory system" [4]. In consideration of the crucial involvement of innate and adaptive immune responses in post-nerve injury, neuropathic pain is currently regarded as a neuro-immune disorder [5].

Inflammation is a pathophysiological state that is usually associated with pain. The activation of glial cells (microglia and astrocyte) in the spinal cord level initiates and maintains the pain hypersensitivity after a nerve injury [6–9]. Peripheral alterations

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involve activating the sensory neurons, including the immune-like glial cells in the injured nerve, the dorsal root ganglia, and the spinal cord, thereby leading to the release of both anti-inflammatory and pro-inflammatory cytokines. Anti-inflammatory cytokines serve as a negative-feedback regulator to maintain a balanced immune response. IL-10 is the most powerful anti-inflammatory cytokine that can encode for pro-inflammatory cytokines, suppress their translation, and down-regulate their receptors. An early increase in IL-10 expression is observed 1 h after a peripheral injury in the sciatic nerve. However, this increasing trend appears to be gradual over a long time and peaks after 6 weeks [5]. Pro-inflammatory cytokines (tumor necrosis factor alpha, TNF-α; interleukin (IL)-6; and IL-1β) in the spinal cord can augment the central sensitization, induce pain hypersensitivity, and participate in the pathogenesis of neuropathic pain [10,11]. Despite the numerous studies, the treatment of neuropathic pain remains one of the most difficult clinical challenges.

Conventional pharmaceutical therapies for neuropathic pain, such as non-steroidal anti-inflammatory drugs (NSAIDs), opioid analgesics, tricyclic antidepressants, and anticonvulsants, are not satisfactory due to their various adverse effects, withdrawal syndromes, extensive limitations, and multiple pathological mechanisms [12]. Particularly, pregabalin (Lyrica) is an anti-epileptic drug widely used to clinically treat neuropathic pain [13,14]. Its analgesic effect on neuropathic pain was observed in a variety of randomized, placebo-controlled clinical trials. Preclinical trials showed that pregabalin possesses anti-allodynic and anti-hyperalgesic effects on neuropathic pain in various animal models [15,16]. Therefore, pregabalin was used as the positive control drug in this study. However, its side effects (dizziness, somnolence, and peripheral edema) seriously affected the treatment and thus should be replaced in future studies.

Chinese herbs received increasing attention due to their abundant resources and long history of safe use in humans. *Glycyrrhiza uralensis* Fisch (Leguminosae) is a traditional Chinese herb that can be traced back to Shen Nong herbs and is commonly used to treat injury or swelling because of its life-enhancing properties and detoxification in traditional oriental medicine. Liquiritin (LQ, C21H22O9) (Fig. 1) is one of the flavonoids extracted from *Glycyrrhizae* radix that shows a variety of pharmacological activities and potential applications, such as preventing inflammation and relieving pain, cough, and allergic reactions [17,18]. LQ exhibits anti-inflammatory, anti-oxidant, neuroprotective, anti-cancer, and antiviral activities [19,20] and exerts a neuroprotective effect against focal cerebral ischemia/reperfusion induced by middle cerebral artery occlusion [21]. Furthermore, Yang et al. [19] reported that LQ has neuroprotective and neurotrophism effects against primary cultured hippocampal cells. However, the neuroprotective effect of LQ against neuropathic pain is not yet reported.

The present study investigated the protective effect of LQ on neuropathic pain using chronic constriction injury (CCI) mouse model to explore the possible involvement of inflammation.

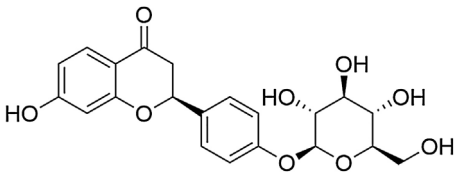


**Fig. 1.** The chemical structure of Liquiritin (LQ, C21H22O9).

## 2. Materials and methods

### 2.1. Experiment animals

Institute of Cancer Research (ICR) mice weighing 18–22 g were obtained from the Experimental Animal Center of Ningxia Medical University (certificate number was SYXK Ningxia 2016-0001).

The mice were housed in an environment with controlled temperature (22 °C–24 °C) and humidity (45%–65%) and 12 h light–12 h dark cycles and had free access to food and water. The experiments were performed in accordance to both the National Guidelines for the Care and Use of Laboratory Animals and the Guidelines for the Care of Laboratory Animals in Ningxia Medical University issued by the Animal Experimental Committee. We also complied with the ethical regulations on animal research.

### 2.2. Drugs and chemicals

LQ (purity ≥ 98.0%) was purchased from Beijing Zhongke Quality Inspection Biotechnology Co., Ltd. (lot no. 151211) and was suspended in sodium carboxymethyl cellulose (0.5% CMC-Na). Pregabalin (Pfizer Manufacturing Deutschland GmbH, Betriebsstatte Freiburg) and sodium pentobarbital (Sigma- Aldrich, Steinheim, Germany) were dissolved in a saline solution (0.9% NaCl). Sodium pentobarbital was injected intraperitoneally (i.p.), whereas the other drugs were injected intragastrically (i.g.) in a ratio of 10 μL/g body weight and were freshly prepared prior to the experiments.

### 2.3. Induction of peripheral neuropathy by CCI

Neuropathic pain was induced by CCI of the sciatic nerve in experimental animals as performed according to the method described by Bennett and Xie [22] with slight modification. The mice were anesthetized with sodium pentobarbital (0.8%, i.p.). The hair on the lower back thigh area of the right paw the mice was shaved, and the skin was sterilized with povidone–iodine. The right sciatic nerve was exposed and isolated from the surrounding tissues. Four chromic gut (silk 4-0) sutures were tied loosely around the sciatic nerve that is close to the bifurcation; 1 mm spacing was maintained between each ligature to reveal a slight flick of the ipsilateral hind limb. Finally, the muscular and skin layer was immediately sutured, and a topical antibiotic was immediately applied. Similar procedure was performed without ligation on mice in the sham-operated controls [23]. All surgical procedures were conducted under normal sterile conditions by one individual.

### 2.4. Experimental protocol

Eighty mice were randomly divided into 8 equal groups of ten mice each as shown in Table 1.

The doses for LQ treatment were based on our preliminary experiments and references [18]. Drug delivery was not performed until day 8 of post-CCI surgery to guarantee establishment of the neuropathic pain model, which was characterized by maximum mechanical allodynia and thermal hyperalgesia. Drugs were administered once a day until the 14th day for each of the experimental groups. Behavioral tests/observation were performed before surgery (day 0) and on days 7, 8, 10, 12, and 14 after CCI, whereas the electrophysiological activities of the sciatic nerve were recorded immediately after these tests on the last day. All mice were sacrificed for further studies, i.e., histopathological evaluation (hematoxylin–eosin (H&E) staining and transmission electron analysis), immunofluorescence, and Western blot analysis.