Identification and potential mechanism of different components from the aerial part of *Bupleurum chinense* DC. for epileptic treatment

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**ABSTRACT**

The present study aimed to assess the effects of epileptic treatment of the aerial part of *Bupleurum chinense* DC. in kainic acid (KA)-induced epilepsy rats and LPS-induced BV2 cells, as well as to identify the active chemical constituents. The *in vivo* and *in vitro* results showed that 20% ethanol elution fractions of the aerial part of *B. chinense* DC. (BCE-20) and 70% ethanol elution fractions of the aerial part of *B. chinense* DC. (BCE-70) could improve the epileptic state of the rats and status epilepticus (SE%). Moreover, ultra-high-performance liquid chromatography (UPLC)-Orbitrap mass spectrometry (MS) analysis identified BCE-20 and 70 as flavonoids and phenylpropanoids, respectively. The mechanistic analysis also showed that BCE-20 and 70 could regulate neurotransmitter abnormalities and suppresses the expression and secretion of pro-inflammatory cytokines. Notably, BCE-20 and 70 could regulate the Triggering receptor expressed on myeloid cells 2 (TREM2)/nuclear factor-k-gene binding (NF-\(\kappa\)B)/inhibitor of NF-\(\kappa\)B \(\alpha\) (I\(\kappa\)B\(\alpha\)) pathway to inhibit the neuroinflammation. Our findings support the ethnopharmacological use of the constituent polyphenols and flavonoids from the aerial part of *B. chinense* DC., as the strong anti-epileptic agents.

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1. Introduction

Epilepsy was the second most common disease of the nervous system, affecting approximately 0.5–1% of the population worldwide, with greater drug resistance and worse clinical outcomes than many other neurological diseases (Kundap et al. 2020). It was particularly important to study the essence of epilepsy, to seek new targets of anti-epileptic drugs (AEDs), and to prevent the progression of epilepsy. Traditional Chinese medicine played a very important role in the treatment of epilepsy with its multi-component, multi-pathway, and multi-target mode, which had obvious characteristics and advantages. Compared with chemical drugs, traditional Chinese medicine had the characteristics of unique curative effects, small side effects, and abundant resources, and was attracting extensive attention at home and abroad.

The aerial parts of *B. chinense* DC. have been used for anti-inflammatory, anti-influenza B virus, and antipyretic in folk medicine in some areas of southeast China and Spain. Recently, it has been reported that the extract of the aerial parts of *B. chinense* DC. (BCE) had good anti-epileptic activity, but its active components were not clear (Kuang et al. 2009; Zhou et al. 2021). However, most of the scientific evaluation on *B. chinense* DC. had been carried out only with the roots. Also, to the best of our knowledge, the anti-epileptic activity and mechanism(s) of action of its aerial parts had been reported only sporadically. The limited amount of information on the anti-epileptic effects of the aerial parts of *B. chinense* DC. and its active constituent(s) prompted our presence. Therefore, this study was designed to separate and identify phytochemicals in the aerial part of *B. chinense* DC. and determine its anti-epileptic activity and potential mechanism.

2. Results and discussion

To determine the composition of the aerial part of *B. chinense* DC., it was first separated. Then, under the same conditions, the UPLC-Orbitrap-MS base peak ion (BPI) chromatograms in the negative ion mode of the BCE-20, 70 and 95% ethanol elution fractions of the aerial part of *B. chinense* DC. (BCE-95) (Figure S1, Supplementary material). According to the comparison of chromatographic peak time, the chemical components of each component have no obvious cross and conduct UPLC-MS full scan, collect data with Mmarkerlynx™ software, and process it through principal
component analysis (Figure S2). As can be seen from the figure, various components are concentrated in different coordinate regions and do not intersect each other. Subsequently, we tested the antiepileptic activity of BCE-20, 70, and 95.

KA could induce typical epileptic seizures, it was found that the high-dose group of BCE-20 (BCE-20H, 200 mg/kg, body weight (b.w.), peros (p.o.)) and the high-dose group of BCE-70 (BCE-70H, 200 mg/kg, b.w., po.) could significantly improve the epileptic state of the rats after 28 days of treatment with BCH-20, BCE-70, and BCE-95 (Figure S3A–B). According to the pathological results, the hippocampal CA3 area of epileptic rats had the structural disorder, a large number of pyramidal cells were necrotic, nucleus pyknosis or fragmentation (blue arrow), and more microglia hyperplasia can be seen. BCE-20H and BCE-70H could improve the hippocampal injury caused by epilepsy, but BCE-95 has no obvious effect (Figure S3E). And there was no toxic effect of each elution component when BV2 microglial cells were treated with each elution component 0–1000 µg/µL, and 500 µg/µL BCE-20, 70, 95 could promote BV2 cell growth, and BCE-20 and 70 could reduce LPS induced the production of Nitric Oxide (NO) (Figure S3C and D).

At the same time, according to the UPLC-Orbitrap-MS technique, BCE-20 and 70 were found to be rich in flavonoids and phenylpropanoids, respectively (Figure S4–S5, Table S1–S2). 12 flavonoids were identified as 4’-methoxyl-tricin, diosmetin 7-O-β-glucuronide, quercetin 3-glucuronide, 3’-methoxy isoquercitrin, isoquercitrin, isorhamnetin 3-O-β-D-glucopyranoside-7-O-α-L-rhamnopyranoside, hesperetin 7-O-β-glucoside, hesperetin 5-O-β-glucoside, shamim, nepititin, isorhamnetin-7-O-α-L-rhamnopyranoside, and tricin-7-O-β-D-glucopyranose (Table S1, Supplementary material). And 8 phenylpropanoids were identified as (+)-syringaresinol, gerberinol, 2-(4-methoxyphenyl)ethyl β-D-glucopyranoside, 1-(4-hydroxy-3-methoxy)-phenyl-2-[4-(1,2,3-trihydroxypropyl)-2-methoxy]-phenoxy-1,3-propandiol, (+)-pinoresinol, acutissimalignan B, citrusin C and sesamolin (Table S2, Supplementary material). Quercetin and hesperidin and their derivatives have been reported to have varying degrees of antiepileptic and neuroprotective effects (Kumar et al. 2013; Dhir 2020). However, the lignan in BCE-70 is mostly an analog of syringaresinol and pinoresinol, which has a strong anti-neuroinflammation effect (L. Zhang et al. 2021; Y. Zhang et al. 2021; Bai et al. 2021). Therefore, flavonoids and phenylpropanoids from B. chinense DC. might be the potential effective components of anti-epilepsy.

KA model had been widely accepted in the world as an excellent animal model to imitate human temporal lobe epilepsy. And it could cause abnormalities in neurotransmitters and neuroinflammation (Neumaier et al. 2018). In the MON group, the content of γ-aminobutyric acid type A receptor (GABA AR) in the hippocampus decreased, and the contents of glutamic acid decarboxylase (GAD) 65, GAD67, glial fibrillary acidic protein (GFAP), G protein gated inwardly rectifying K channels 1 (GIRK1), and N-methyl-D-aspartic acid receptor 1 (NMDAR1) increased, BCE-20H and BCE-70H could improve the abnormal release of these neurotransmitters (Figure S6).

Our results also indicated that BCE-20 and 70 could significantly decrease the expressions of Interleukin-1β (IL-1β), Interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) in KA-induced epilepsy rats and suppress the lipopolysaccharide (LPS)-induced production of NO, IL-1β, IL-6, and TNF-α, shown in Figure S7–S8. Excessive NO and
Proinflammatory cytokines may lead to severe diseases such as septic shock, inflammatory diseases, and neurotoxicity (Teeling and Perry 2009). Proinflammatory cytokines upregulate both cyclooxygenase 2 (COX-2) and inducible nitric oxide synthase (iNOS) expression via the NF-κB pathway (Lucas et al. 2006). BCE-20 and BCE-70 showed an excellent inhibitory effect on the expression of iNOS and COX-2, which played an important role in the innate response in activated microglia cells. NF-κB served as a critical mediator of the inflammatory response, and its activation was involved in the early stage of neurodegenerative diseases. The above data showed that BCE-20 and 70 could suppress the expression of pro-inflammatory mediators and cytokines regulated by NF-κB. Therefore, we further investigated the role of flavonoids and phenylpropanoids in the activation of the NF-κB pathway. First, we found that BCE-20 and 70 could suppress the increased phosphorylation levels of NF-κB stimulated by KA and LPS (Figure S9–S10). IκB, which retained NF-κB in an inactive state in the cytoplasm, was one important modulator of the upstream NF-κB signal transduction cascade (Kaltschmidt and Kaltschmidt 2009). BCE-20 and 70 significantly decreased the phosphorylated levels of IκB in KA-induced epilepsy rats and LPS-induced BV2 microglial cells (Figure S9–S10).

Finally, we focused on TREM2, the microglial surface receptor, KA and LPS could decrease the expression of TREM2, but BCE 20 and 70 increased the expression of TREM2 (Figure S9–S10). TREM2 is expressed on the surface of microglia and mediates key functions of microglia, including the removal of dying neurons and inhibition of pro-inflammatory responses, making suppression of microglia abnormal activation a therapeutic strategy for neuroinflammatory diseases (Li et al. 2019). As shown in our study, the release of NO in LPS-induced BV2 was reduced with BCE-20 and 70, and the proinflammatory response was inhibited by the regulation of TREMR 2. Previous studies have shown that TREM2 can mediate the functions of microglia in the central nervous system (CNS), such as clearance of apoptotic neurons and secretion of pro-inflammatory cytokines (Hamerman et al. 2006). Our study has demonstrated that the treatment of BCE-20 and 70 increased the expression of TREM2 protein levels in BV2 microglia. On the other hand, BCE 20 and 70 could regulate TREM2 to abolish NF-κB activity and secretions of TNF-α. These findings suggested that TREM2 played a critical role in transforming microglia from a homeostatic to a disease-associated state. Additionally, TREM2 has been proposed as a new therapeutic target for the treatment of epilepsy. The involvement of TREM2 in the pharmacological functions of BCE-20 and 70 in anti-epileptic activity implicates a novel molecular mechanism. The results above indicated that BCE-20 and 70 could effectively inhibit the activity of the epilepsy-induced TREM/NF-κB/IκB signaling pathway.

3. Conclusion

In this experiment, BCE-20 and 70 indicated a good anti-epilepsy ability in the aerial parts of B. chinense DC. According to the UPLC-Orbitrap-MS technique, BCE-20 and 70 were found to be rich in flavonoids and phenylpropanoids, respectively. Furthermore, flavonoids and phenylpropanoids in the aerial parts of B. chinense DC. could inhibit epilepsy-induced inflammatory responses including decreasing the production of...
inflammatory mediators, suppressing the expression of iNOS and COX2, and inhibiting the secretion of pro-inflammatory cytokines (TNF-α, IL-6, and IL-1β) in epilepsy rats and BV2 microglial cells. In addition, flavonoids and phenylpropanoids reduced TREM/NF-κB/IκB pathway activation in epilepsy-activated microglial cells. Collectively, these results suggested flavonoids and phenylpropanoids in the aerial parts of B. chinense DC. potentially inhibited neuroinflammation and might benefit the treatment of epilepsy.

Disclosure statement
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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