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Original Article

Experimental Neurobiology

Siegesbeckia Orientalis L. Extract Attenuates Postoperative Cognitive Dysfunction, Systemic Inflammation, and Neuroinflammation

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A proportion of patients experience acute or even prolonged cognitive impairment after surgery, a condition known as postoperative cognitive dysfunction (POCD). It is characterized by impairment in different cognitive domains and neuroinflammation has been implicated as one of the inciting factors as strategies targeting inflammation tend to improve cognitive performance. *Siegesbeckia Orientails L.* (*S. Orientails*) is a common Chinese medicinal herb used for managing chronic inflammatory diseases. We investigated if pretreatment with *S. Orientails* before surgery confers any neuroprotective effects in postoperative animals in terms of reducing inflammation and mitigating cognitive impairment. Three-month-old male C57BL/6N mice were fed different doses of *S. Orientails* extract for 14 days before they underwent a laparotomy. After cognitive testing they were sacrificed on postoperative day (POD) 3. Our results showed that animals with extract pretreatment demonstrated memory improvement in a dose-dependent manner compared with control. Further, evidence for the attenuation of systemic and neuroinflammation was found in the pretreated animals, along with the inhibition of inflammatory pathways and significantly reduced tau phosphorylation in the hippocampus. Taken together, these results demonstrated a neuroprotective effect of *S. Orientails* in postoperative animals, indicating a therapeutic potential of *S. Orientails* in minimizing POCD and the possibility of utilizing this traditional Chinese medicine perioperatively.

Key words: Surgery, Cognitive dysfunction, inflammation, Tau

INTRODUCTION

Postoperative cognitive dysfunction (POCD) is a clinical condition that is characterized by impairment in multiple cognitive domains including memory, concentration, language comprehension and learning difficulties [1]. Although acute and mild cognitive decline commonly occurs after surgery, some patients develop more severe and enduring forms of cognitive dysfunction that maybe associated with increased mortality [2]. The underlying

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mechanism of POCD is still unclear but postoperative neuroinflammation has been shown to be involved with the development of this condition [3]. Previous studies have demonstrated that both surgical trauma and anesthesia could initiate systemic and neuroinflammation in postoperative models [3]. For instance, the general anesthetic agent isoflurane up-regulates pro-inflammatory cytokines brain of mice [4]. Macrophages penetrate the brain and trigger glia activation and up-regulates inflammatory cytokines in the hippocampus after surgery [5, 6]. This was associated with cognitive impairment but the degree of dysfunction was reduced by inhibiting macrophages activation and neuroinflammation in the brain [5]. Neuroinflammation also plays a critical role in synaptic dysfunction and tauopathy, in which tau hyperphosphorylation is observed under neuroinflammatory states [7]. Abnormal tau phosphorylation and accumulation are shown to interfere with synaptic function and results in cognitive impairment [7]. Collectively, these data highlight the pivotal role of neuroinflammation in the pathogenesis of POCD and the therapeutic potential of anti-inflammatory strategies to minimize POCD by inhibiting inflammation. These strategies include pharmacological pretreatment that renders an organism more resistant to a subsequent significant insult, a concept that is similar to the practice of Traditional Chinese Medicine (TCM) that emphasizes the "preventive measure".

Siegesbeckiae Herba (SH, also called Xixiancao in Chinese) is a traditional Chinese medicine (TCM) commonly used for eliminating symptoms including wind-damp, limbs weakness and detoxification as first recorded in the *Newly Compiled Materia Medica*. Being one of the main plant sources of SH, the dried aerial part of *Siegesbeckia Orientails* L. (*S. Orientails*) is usually used for management of chronic inflammatory diseases such as rheumatoid arthritis (RA). In the last few decades, numerous studies have focused on the anti-inflammatory effect of *S. Orientails* and/or its derivative compounds in experimental RA models, with its use improving both the scores and inflammatory markers of the animal with arthritis [8]. In view of the anti-inflammatory properties of *S. Orientails*, we hypothesized that *S. Orientails* could alleviate postoperative cognitive deficits by attenuating systemic inflammation and neuroinflammation. The purpose of current study is to explore the therapeutic potential of *S. Orientails* in postoperative animals, especially in terms of cognitive performance which is correlates with the presence of inflammation and tau modulation.

MATERIALS AND METHODS

Animals

3-month-old C57BL6/N mice were obtained from The University of Hong Kong. The handling of animal and all procedures were conducted in accordance with National Institutes of Health guide for the care and use of Laboratory animals and Animals (Control of Experiments) Ordinance, Hong Kong, China. The use of animals was approved by the Department of Health, Hong Kong and Committee on the Use of Live Animals in Teaching and Research, The University of Hong Kong. All efforts were made to minimize animal numbers and suffering. In the current report, mice were divided into 6 groups: Sham control (Ctrl), surgery (Lap), low

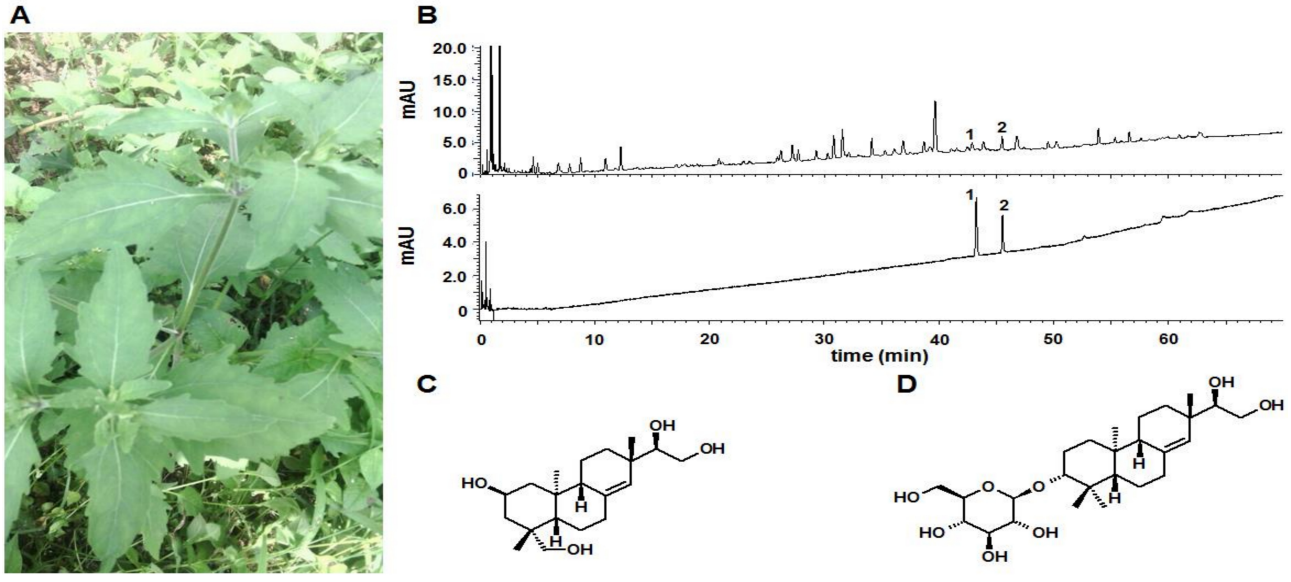

Fig. 1. Preparation and chemical profile of *S. Orientails* extract. (A) Pictures of *S. Orientails L.*. (B) Chemical profile of *S. Orientails* analyzed by UPLC. 1, Kerinol; 2, Darutoside. (C) Chemical structure of kerinol. (D) Chemical structure of darutoside.

dose drug control (0.75 g/kg/day), high dose drug control (1.5 g/kg/day), low dose drug with surgery (0.75 g/kg/day plus Lap) and high dose drug with surgery (1.5 g/kg/day plus Lap).

Preparation and characterization of S. Orientails L. extract

The herb of *S. Orientails* (Fig. 1A) was collected from Gubao Town (Xiuwen County, Guiyang, Guizhou Province, China) (time of collection: August, 2015), and authenticated by the corresponding author, Dr. Hua YU. The voucher specimens (No. SO-002) were deposited at the Institute of Chinese Medical Sciences, University of Macau, Macao, China.

For use in the experiments, a powdered SO (100 g) was reflux-extracted twice with 10-fold volume of 50% ethanol (v/w) for 1 hr for each. The combined extract was cooled, filtered, and then concentrated under reduced pressure to remove the ethanol. Subsequently, the concentrated extract was lyophilized with a Virtis Freeze Dryer (The Virtis Company, New York, USA). The powdered *S. Orientails* extract (yield: 15.4%, brown color) was kept at 4°C for further experiments.

Quantifications of two major active compounds (kirenol and darutoside) in the *S. Orientails* extract was performed using a Waters ACQUITY-UPLC CLASS system (Waters Corp., Milford, USA) coupled with an ACQUITY UPLC HSS T3 column (150 mm×2.1 mm, 1.8 μm) maintained at 40°C. Elution was performed with a mobile phase of A (0.2% H3PO4 in water) and B (0.2% H3PO4 in ACN) under a gradient program: 0~5 min, 17% B; 5~15 min, 17%~25% B; 15~30 min, 25~50%. The flow rate was 0.4 ml/min and the injection volume was 2 μl. The analytes were monitored at the UV wavelength of 215 nm. Between two injections, the column was washed with 100% B for 2 min and equilibrated with the initial mobile phase for 5 mins.

Surgical and drug treatments

To examine if *S. Orientails* extract ameliorates postoperative cognitive dysfunction, mice assigned to drug treatment groups were given the agent for 14 days prior to surgery. Timeline of whole experiment is shown in Fig. 2A. The extract was dispersed in 1X PBS and 100 μl of extract solution was orally administrated to mice at the dosages of 0.75 g/kg/day and 1.5 g/kg/day for 14 consecutive days. Sham control and surgery groups were given equivalent

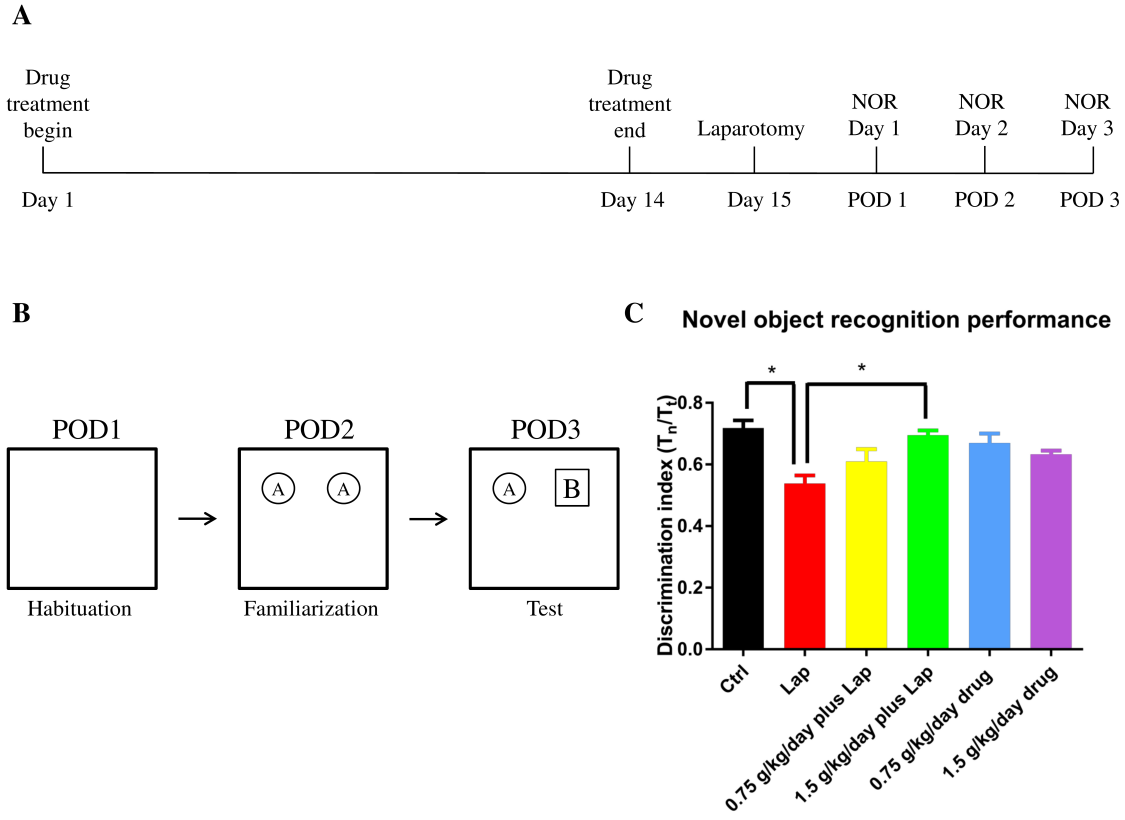

Fig. 2. *S. Orientails* improved hippocampal dependent memory function in postoperative animals in dose-dependent manner. The mice demonstrated impaired object recognition memory function on postoperative day 3. (A) Timeline of the drug treatment and behavioral test. (B) Schematic diagram for novel object recognition test. (C) A shorter novel object exploration time was observed in the surgical group while *S. Orientails* extract increased exploration time of the novel object. Data are presented as the mean and SEM (n=6). *p<0.05 between groups.

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amount of 1X PBS. On day 15 the mice underwent a midline laparotomy under 3% sevoflurane general anesthesia in 100% oxygen. After incision, the gastrointestinal tract was exteriorized and rubbed by fingers for 1 min to mimic surgery, after which it was replaced into the peritoneal cavity followed by wound closure. The mice were allowed to recover before further testing.

Novel object recognition test

Hippocampal dependent object recognition memory was determined by the novel object recognition (NOR) test as described before but with modifications [9]. Briefly, on postoperative day (POD) 1, mice were put in an open field box (50×50 cm) with no objects for 10 mins for habituation. On POD 2, mice were placed in the same box with 2 identical objects for 10 mins for familiarizing the object. Twenty-four hours after familiarization on POD 3, one of the two objects was replaced with a novel object. The mice were allowed to stay in the box for 10 mins and the behavior of the animals was video recorded. Schematic diagram is shown in Fig. 2B. Object exploration was scored by the amount of time with the nose pointed towards and located within 2 cm of the object. The discrimination index was calculated by the formula: T_n/T_t , where T_n is the time of exploring novel object while T_t is the total time of exploring novel and familiarized object. After completing the novel object recognition test, the mice were sacrificed by CO_2 asphyxiation. 1 ml of blood was extracted by cardiac puncture and serum was isolated after 1,300 g centrifugation for 10 mins. Liver and hippocampal tissues were then dissected out from animals for subsequent real time PCR and Western Blot experiments.

mRNA extraction and real time PCR

Hepatic and hippocampal mRNA were isolated by RNAiso plus (Takara, Japan). Tissues were first homogenized in RNAiso plus and vigorously mixed with chloroform. After 10000 g centrifugation at 4°C, the upper aqueous layer was isolated. mRNA pellets were precipitated by isopropanol and washed with 75% ethanol. It was then dissolved in diethyl pyrocarbonate (DEPC) treated water. 1μg of mRNA was converted to complementary DNA sequence by reversed transcription using cDNA synthesis kit according to manufactures' protocol (Takara, Japan). Levels of cDNA of different inflammatory cytokines were assessed by real time PCR with respective primers: 1) IL-1β, forward: CCTCCTTGCCTCTGATGG, reverse: AGTGCTGCCTAATGTCCC; 2) IL-6, forward: TTCACAAGTCCGGAGAGGAG, reverse: TCCACGATTTCCCAGAGAAC; 3) IL-8, forward: TGCCGTGACCTCAAGATGTGCC, reverse: CATCCACAAGCGTGCTGTAGGTG; 4) TNF-α, forward: CCCCAGTCTGTATCCTTCT, reverse: ACTGTCCCAGCATCTTGT; 5) GAPDH, forward: ATTCAACGGCACAGTCAA, reverse: CTCGCTCCTGGAAGATGG.

Measurement of serum inflammatory markers

Inflammatory cytokine expression in the serum was evaluated by MILLIPLEX MAP mouse cytokine/chemokine magnetic bead panels (EMD Millipore Corp., Billerica, MA). All primary data points were collect on a Luminex MAGPIX system. Protein samples and detection substrates were incubated on the plate with specific antibodies-conjugated magnetic beads coated on each well. Fluorescence signal detection and analysis were performed according to the manufacturer's protocol. Concentrations of IL-1β, IL-6, MIP-2 (homologues of IL-8) and TNF-α in serum were determined.

Western blot

Hippocampal tissue was dissected out and homogenized as mentioned above. Proteins were extracted with RIPA lysis buffer (Cellsignal, Danvers, MA) supplemented with protease and phosphatase inhibitors (Roche, Berlin, German). Protein samples were quantified by BCA protein assay. Proteins were resolved by SDS-PAGE gel and transferred to PVDF membrane. After blocking with 2% non-fat milk, membranes were probed overnight at 4°C with different primary antibodies (JNK, p-JNK, p65 and p-p65 were from Cellsignal, MA; P-tau S396 and β-actin were from Thermo Fisher Scientific, MA; Pan-tau was from DAKO, Japan), followed by respective HRP-conjugated secondary antibodies for 1 hr. Protein bands were visualized by enhanced chemiluminescence (ECL) reagents and signals were captured by X-ray film. The intensities of protein bands were quantified by Image J analysis software.

Statistical analysis

Comparison between different groups of treatment was analyzed by one-way ANOVA with Turkey post-hoc test. Significant differences were considered between groups when $p<0.05$.

RESULTS

Characterization of S. Orientails extract

Kerinol (Fig. 1C) and darutoside (Fig. 1D) are the two most important compounds in *S. Orientails*, both demonstrating anti-inflammatory effects *in vitro* and *in vivo* [10, 11]. Moreover, kerinol is the recommended chemical marker use for quality control of the *S. Orientails* herb by the Chinese Pharmacopeia. Therefore, quantifications of kerinol and darutoside in *S. Orientails* extract were performed. As illustrated in Fig. 1B, kerinol and darutoside

can be chromatographically separated using the developed UPLC method. The contents of kerinol and darutoside in the in *S. Orientails* extract were determined to be 0.24±0.01% and 1.47±0.11%, respectively.

S. Orientails extract ameliorated cognitive impairment in postsurgical animals in a dose dependent manner

Memory deficits on POD 3 were observed in the surgical group as manifested by a significant reduction of novel object exploration time (Fig. 2). Drug treatment alone did not exert any adverse effects on memory function. In the surgery plus drug groups, although no significant improvement of cognitive deficit was found with the low dose (0.75 g/kg/day) treatment, the high dose (1.5 g/kg/day) treatment significantly improved memory function as reflected by the increase in the discrimination index (Fig. 2, p=0.0265 between Ctrl and Lap; p=0.0376 between Lap and 1.5g/kg/day plus Lap). No significant differences were found between other groups; p>0.05). These data demonstrated that pretreatment with the extract improved postoperative memory function in dose dependent manner.

S. Orientails pretreatment improved both systemic and neuroinflammation in postoperative animals

To investigate if extract pretreatment could attenuate inflammatory responses in postoperative animals, mRNA of pro-inflammatory cytokines in the liver were examined by real time PCR. Significant up-regulation of IL-6 was observed in the laparotomy group while high dose extract pretreatment ameliorated the increased mRNA expression of these pro-inflammatory cytokines (Fig. 3A) (p=0.0117 between Ctrl and Lap; p=0.0127 between Lap and 1.5 g/kg/day plus Lap, non-significance was found between other groups; p>0.05). In addition, cytokine levels in the serum were examined by Milliplex assay. In line with the results from real time PCR, serum concentration of IL-6 (p=0.015 between Ctrl and Lap;

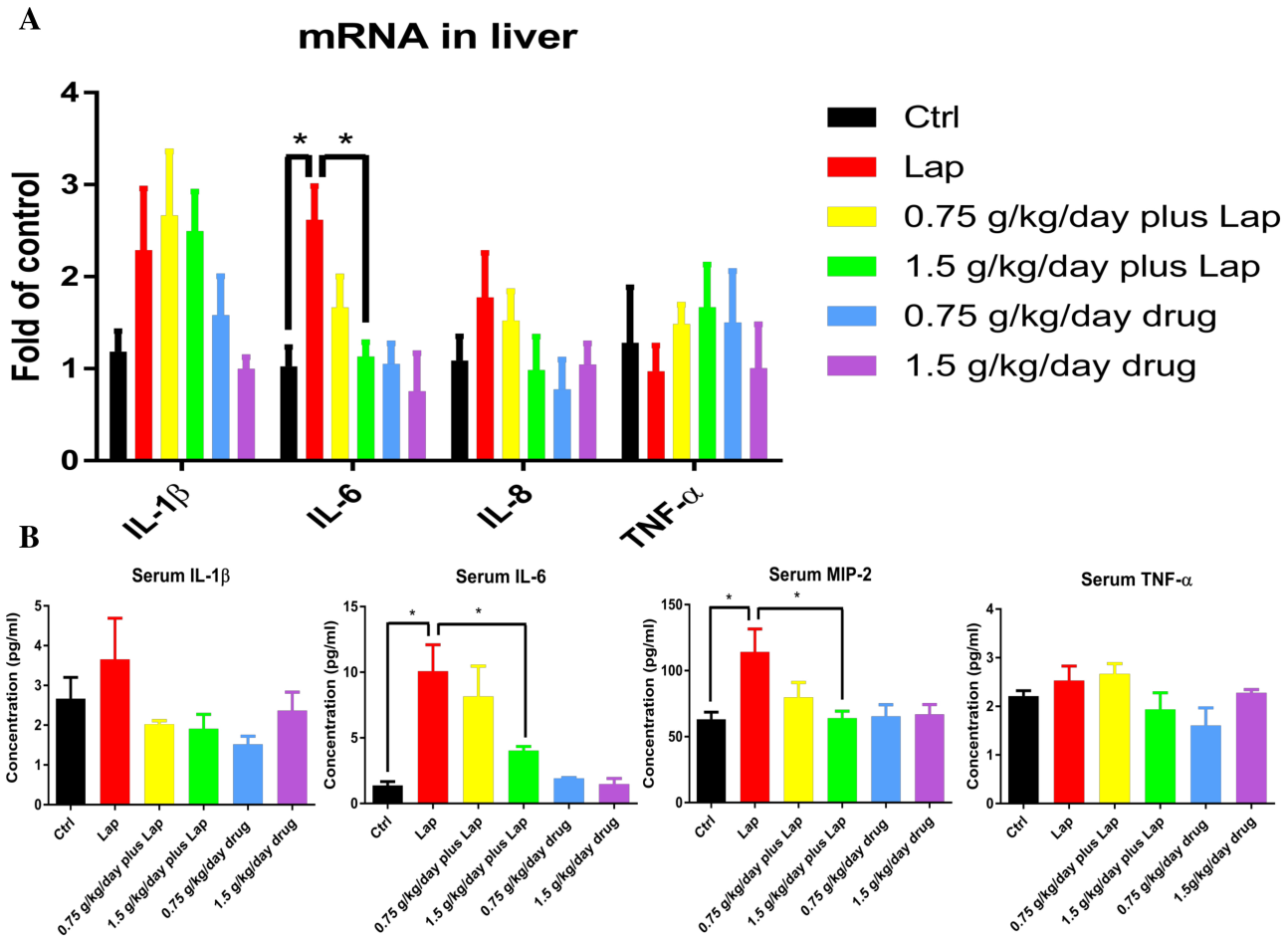

Fig. 3. *S. Orientails* inhibited surgery induced systemic inflammation. Laparotomy increased pro-inflammatory cytokine gene expressions (A) in the liver and (B) level of inflammatory cytokines in serum. Postoperative mice received extract pretreatment inhibited the systemic inflammatory responses. Data correspond to the mean and SEM (n=4-5). GAPDH was used as mRNA internal control. *p<0.05 between groups.

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p=0.044 between Lap and 1.5 g/kg/day plus Lap, non-significance was found between other groups; p>0.05) and MIP-2, the homologue of IL-8, were significantly increased in surgery group while reduced after high dose extract treatment (Fig. 3B. p=0.0137 between Ctrl and Lap; p=0.0236 between Lap and 1.5 g/kg/day plus Lap, No significant difference was found between other groups; p>0.05) Finally, to examine the neuroinflammatory response, mRNA expression of inflammatory cytokines were examined by real-time PCR. Increase in IL-1β (p=0.0337 between Ctrl and Lap; p=0.0416 between Lap and 1.5 g/kg/day plus Lap, no significant difference between other groups; p>0.05) and IL-6 (p=0.0339 between Ctrl and Lap; p=0.0482 between Lap and 1.5 g/kg/day plus Lap, no significant difference between other groups; p>0.05) were observed in the hippocampi of the surgical group. These increases were attenuated by high dose extract (Fig. 4). Overall, these results demonstrated that the extract reduced inflammation in both peripherally and centrally.

Pro-inflammatory JNK and NF-κB pathways were inhibited by S. Orientails extract

After observing the inflammatory response in the hippocampus, we further examined if extract pretreatment inhibited neuroin-

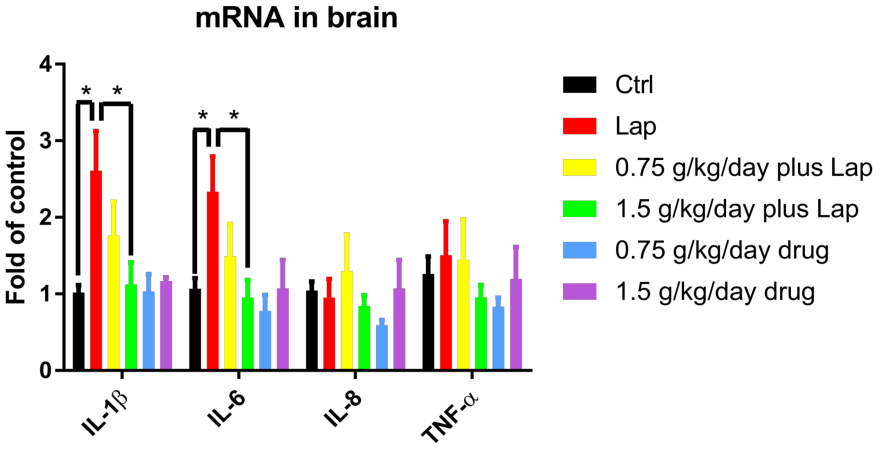

Fig. 4. *S. Orientails* reduced neuroinflammation in the hippocampus induced by surgery. Neuroinflammatory response was evaluated in the hippocampus by examining inflammatory cytokines gene expression. Data correspond to the mean and SEM (n=4-5). GAPDH was used as mRNA internal control. *p<0.05 between groups.

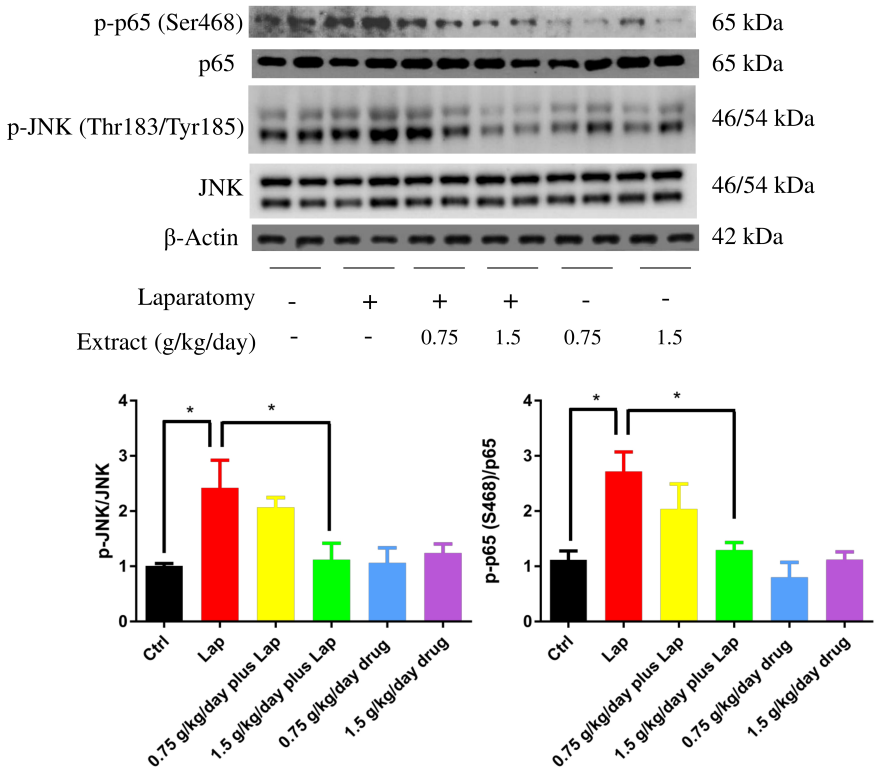

Fig. 5. *S. Orientails* inhibited activation of JNK and NF-κB pathways in the hippocampus in postoperative animals. Activation of JNK and NF-κB pathways in the hippocampus by laparotomy while pretreatment of *S. Orientails* extract inhibited the activities of both pathways. Representative blots against phosphorylated JNK and p65 in the hippocampus of different groups of animals. Graphs below showing quantification of western blot. Data correspond to the mean and SEM (n=4) and represents the band densities that were normalized with endogenous JNK and p65 respectively. *p<0.05 between groups.

flammation through modulating pro-inflammatory pathways (JNK and NF-κB) by measuring phosphorylation of JNK and p65 subunit using Western blot. In postsurgical animals, significant up-regulation of phosphorylated JNK (p=0.0226 between Ctrl and Lap; p=0.0403 between Lap and 1.5 g/kg/day plus Lap, non-significance was found between other groups; p>0.05) and p65 (p=0.0182 between Ctrl and Lap; p=0.021 between Lap and 1.5 g/kg/day plus Lap, non-significance was found between other groups; p>0.05) was observed in the hippocampus, whereas extract pretreatment reduced the phosphorylation of both molecules in a dose dependent manner (Fig. 5). These results imply that extract pretreatment may attenuate neuroinflammatory responses through suppressing JNK and p65 activities and subsequent nucleus translocation in the hippocampus.

Tau phosphorylation in the hippocampus was reduced after S. Orientails extract pretreatment

Neuroinflammatory response is closely related to tau phosphorylation, which leads to the destabilization of tau from microtubules and subsequent memory deficits [7]. To examine if surgery induces tau phosphorylation in the hippocampus and whether extract pretreatment impedes this process, protein samples were subjected to Western blotting and examined by phosphorylated tau antibody at Serine 396 epitome. Significant up-regulation of tau phosphorylation was found in the hippocampus after surgery, while extract pretreatment reduced tau phosphorylation (Fig. 6) (p=0.0159 between Ctrl and Lap; p=0.0268 between Lap and 1.5 g/kg/day plus Lap, non-significance was found between other groups; p>0.05). These results indicated that extract may provide a neuroprotective effect to postoperative animals through reducing neuroinflammation and tau phosphorylation.

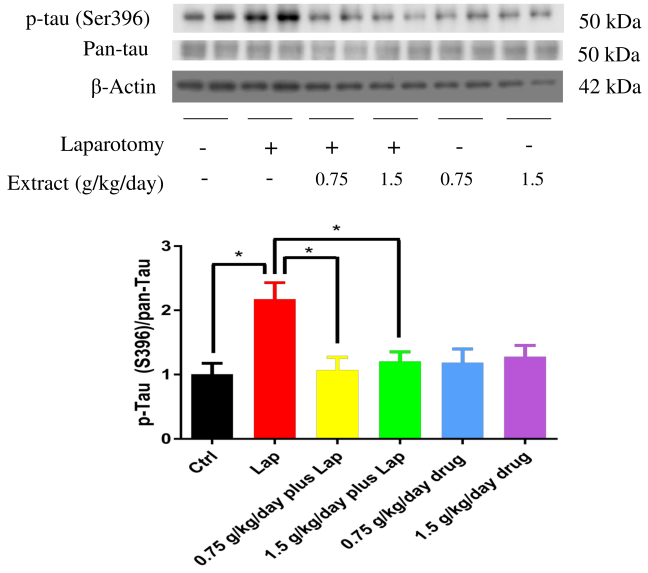

Fig. 6. *S. Orientails* reduced phosphorylation of tau in the hippocampus induced by surgery. Increased tau phosphorylation was observed in the hippocampus of postoperative mice while *S. Orientails* extract pretreatment reduced tau phosphorylation. Graphs below showing quantification of Western blot. Data correspond to the mean and SEM (n=4) and represents the band densities that were normalized with total tau (pan-tau). *p<0.05 between groups.

DISCUSSION

Substantial evidence suggest that surgery induces cognitive dysfunction in patients in whom a continuous inflammatory response is present [3]. Apart from various physical barriers, perioperative inflammation was once considered as the second line of defense mechanism to protect human from pathogens after surgery. Nevertheless, it is increasingly recognized that several postoperative complications are related to inflammation including atrial fibrillation [12], hemorrhage [13] and cognitive dysfunction [14]. Various therapeutic strategies targeting inflammation throughout the surgical period have been suggested to combat these complications. In agreement with previous reports, our results suggest that surgery induces systemic and neuroinflammatory response in postoperative animals [3]. Furthermore, activation of pro-inflammatory pathways was observed, which was associated with an increase in tau phosphorylation and cognitive impairment, thus showing that treatment using *S. Orientails* extract before surgery partially prevented these pathological changes and demonstrated the therapeutic potential of *S. Orientails* in preventing POCD.

It is well established that peripheral inflammation could trigger a neuroinflammatory response in the brain [15]. Chronic neuroinflammation has been shown to accompany deterioration in cognitive function. For instance, inflammation cytokines induce the hyperactivation of astrcotyes [16], which may trigger the release of other cytokines or gliotransmitter such as GABA that affect neuronal activity and plasticity in the AD model [17]. Inhibiting neuroinflammation in turn resulted in amelioration of astrogliosis and improved subsequent cognitive impairment in AD [18]. On the other hand, therapeutic strategies targeting systemic inflammation may improve neuroinflammation and ameliorate neuronal damage, synaptic dysfunction and cognitive performance in different models, including POCD [19]. Traditional Chinese medicine has been used over many centuries and mounting evidence has demonstrated anti-inflammatory properties. *S. Orientails* has been widely used in treating arthritis through inhibiting systemic inflammation [20, 21]. From previous studies, it was shown to improve paw edema in experimental RA animal model, accompanied with the reduction of IL-6 in serum [22].

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From *in vitro* assay, *S. Orientails* was shown to reduce IL-6, TNF-α and NO in RAW264.7 cells. Topical application of *S. Orientails* active components reduces inflammatory cytokines in carrageenan and complete Freund's adjuvant induced inflammatory models [23]. These data demonstrated that *S. Orientails* is a potent natural anti-inflammatory herb which may exert broad range of beneficial effects by ameliorating inflammatory responses.

In view of these anti-inflammatory properties, we examined whether extract from *S. Orientails* can improve the cognitive function in postoperative animal. We have shown a significant increase in the novel object exploration time in postoperative animals with 14 days of extract pretreatment compared with surgery only group, implying an improvement in hippocampal dependent recognition memory. Based on this observation, we examined if the extract could reduce systemic inflammation and neuroinflammation in postoperative mice. Along with better preservation of cognitive function, there was a reduction of pro-inflammatory cytokines IL-6 and IL-8 in the liver and serum of postoperative animals with extract pretreatment in a dose dependent manner (Fig. 3). At the same time, extract pretreatment reduced the mRNA of IL-1β and IL-6 in the hippocampus (Fig. 4). These results demonstrated that extract pretreatment reduced inflammatory responses by suppressing pro-inflammatory cytokines in both the periphery and in the hippocampus, a result similar to that found in experimental RA models.

To further elucidate how the extract could reduce the production of pro-inflammatory cytokines in brain, we investigated if the activation of pro-inflammatory intracellular signaling was modulated. JNK and NF-κB are major pathways involve in inflammation [24, 25] and they respond to external stimulus such as reactive oxygen species, bacteria or virus. In neurodegeneration, activation of JNK and NF-κB are observed in the brains of Alzheimer's Disease models [26, 27]. Both molecules are phosphorylated and translocated to the nucleus, binding to the promoter and enhancing the transcriptional activities of the genes, thus increasing the synthesis of pro-inflammatory cytokine [24, 25]. Over-activation of JNK and NF-κB in the brain are shown to worsen inflammatory responses while inhibition of these molecules ameliorates neuroinflammation and subsequent cognitive dysfunction. Our results demonstrated that surgery leads to the phosphorylation of both JNK and NF-κB in the brain, which was in line with the increase in pro-inflammatory cytokine gene expression. In contrast, extract pretreatment significantly reduced the phosphorylation of both JNK and NF-κB (Fig. 4), thus further confirming that the extract reduces the neuroinflammatory response by impeding the activation of pro-inflammatory pathways in the hippocampus.

One of the possible explanations for the anti-inflammation effect of *S. Orientails* is the modulation of peripheral immune activities. Previous reports have shown that surgery-induced neuroinflammation was partially triggered by the activation and penetration of peripheral immune cells into the central nervous system [28]. These cells could activate inflammatory pathways and stimulate the production of inflammatory cytokines in the brain. On the other hand, previous studies also indicated that *S. Orientails* could modulate the activities of peripheral immune cells [29, 30], which implies the that *S. Orientails* may also inhibit surgery-induced neuroinflammation through regulating peripheral immunity. However, apart from interlukins, we could not observe significant modulation of other cytokines such as TNF-α in the brain. It may be accounted by the differential expression time of respective cytokines. For example, from *in vitro* studies, up-regulation of TNF-α mRNA was observed in lipopolysaccharide (LPS) challenged bone marrow derived dendritic cells as early as 1 hr and returning to basal levels within 24 hrs [31], which may explain the failure of observing TNF-α modulation in our current study. It needs further investigation to confirm if other cytokine expression will be modulated at earlier time points in the postoperative period.

We further asked how the attenuation of neuroinflammation by the extract may modulate tau phosphorylation in the hippocampus. Tau is an important microtubule associated protein in which abnormal phosphorylation and aggregation of tau protein is a pathological hallmark in cognitive dysfunction model [7]. Upon phosphorylation, tau was shown to be dissociated from microtubule which undergo aggregation and oligomerization and exert neurotoxicity or inhibit the function of synapse, leading to synaptic and neuronal dysfunction. It has been shown that inflammatory cytokines such as IL-6 could induce tau phosphorylation which deteriorate neuronal degeneration [32]. In our model, tau phosphorylation was up-regulated in the hippocampus after surgery. With extract pretreatment , reduction of phosphorylated tau was observed compared with surgery alone group (Fig. 5). These results imply that *S. Orientails* extract may have beneficial effect on postoperative animals through anti-inflammation and a reduction in the tau phosphorylation burden in the hippocampus, which is consistent with the previous findings that reducing tau phosphorylation improved neuronal and synaptic deficit in neurodegeneration such as Alzheimer's disease [33]. For future studies, other tau phosphorylation sites and the aggregation of tau protein in the hippocampus maybe required to give a full spectrum of how *S. Orientails* can modulate tauopathy in postoperative animals.

Despite the promising effect of *S. Orientails* in the current study, some issues would still need to be addressed. For example, pulmonary toxicity was reported for the water extract of Herba Siegesbeckiae (HS) in mice [34], and the toxic compounds were

identified to be presented mainly in the aqueous-soluble part of the extract [35]. The median lethal dose (LD50) for oral acute toxicity of the water extract of HS in mice was reported to be 146.7 g (herb)/kg, and the toxic effects was observed when the dosage was higher than 24.4 g (herb)/kg in a 2-week sub-acute test. Moreover, the acute toxicity of the 70% ethanol extracts of HS (LD50: 267.00 g (herb)/kg) was determined to be lower than that of the water extract (LD50: 147.91 g (herb)/kg). Although the maximum dosage of *S. Orientails* was set at 10 g (herb)/kg in this study, which was much lower than the LD50 of the acute and sub-acute dosages of the water extract (LD50: 24.4 g (herb)/kg), the potential toxicity of the *S. Orientails* should be carefully considered and further investigation is needed in future.

To conclude, this study presented *in vivo* data on the neuroprotective effect of *S. Orientails* extract against surgery induced cognitive dysfunction. With pretreatment using higher doses of *S. Orientails* extract, surgery induced systemic and neuroinflammation were ameliorated, as shown by the inhibition of inflammatory pathway JNK and NF-κB and the reduction of interlukin inflammatory cytokines expression respectively. This is associated with the attenuation of tauopathy in the hippocampus that may underlie the neuroprotective mechanism in our model. Taken together, we have demonstrated that *S. Orientails* may have beneficial effect in postoperative subjects.

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