Original Article

The Effects of Aqueous Extracts of *Aconiti ciliare tuber* on Functional Recovery after Sciatic Crushed Nerve Injury in Rats

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Objective : The aim of this study was to evaluate the effects of *Aconiti ciliare tuber* on the descending pain and the recovery of locomotor function that results from sciatic crushed nerve injury in rats.

Method : In order to assess the effects of the aqueous extract of *Aconiti ciliare tuber* on the recovery rate of locomotor function, we investigated the walking track analysis, and for the effects on the pain control we investigated brain-derived neurotrophic factor (BDNF) and inducible nitric oxide synthase (iNOS) expression in the sciatic nerve and on the expressions of c-Fos in the ventrolateral periaqueductal gray (vlPAG) region resulting from the sciatic crushed nerve injury in rats.

Result : Treatment with *Aconiti ciliare tuber* significantly enhanced the SFIvalue, enhanced BDNF expression, decreased iNOS expression, and suppressed c-Fos expression. The present results showed that *Aconiti ciliare tuber* facilitated functional recovery following sciatic crushed nerve injury in rats. The recovery mechanisms of SFI by *Aconiti ciliare tuber* might be ascribed to the increase of BDNF expression for nerve regeneration and reinnervation and to the suppression of iNOS expression for inhibiting nerve inflammation.

Conclusion : In this process it has been shown that *Aconiti ciliare tuber* can be used for pain control and functional recovery from peripheral nerve injury.

Key Words : *Aconiti ciliare tuber*, sciatic nerve injury, sciatic function index (SFI), brain-derived neurotrophic factor (BDNF), inducible nitric oxide synthase (iNOS), c-Fos

Introduction

Peripheral nerve systems are often damaged by crush, compression, ischemia and various diseases. In the sciatic crushed nerve injury, the injured limb displays hyperalgesia, pain-related gait and swelling[1]. These features are abnormal responses to peripheral stimuli and they induce changes of secretion and neural transmission. In peripheral injury, inflammatory reaction by injury induces hyperalgesia[2] and peripheral changes induced by injury also activate various brain regions[3].

After unilateral sciatic nerve injury, rat's gait shows characteristic changes, such as functional loss of extensors or flexors of the foot. This deficit causes the foot to drop to the ground and thus the footprints are altered. Gradual disappearance of these changes reflects nerve regeneration and functional recovery. In this way, footprints can be used to assess sciatic nerve function[4]. De Medinaceli *etal*. suggested the sciatic function index (SFI) first as standard method for measuring the rate of functional

recovery after sciatic nerve injury in rats and the currently used modified SFI formulation was estab-lished by Bain*etal*[5,6]. SFI formula is based on the characteristic walking patterns following sciatic nerve injury in rats and the recovery rate can be determined by this gait analysis.

Nitric oxide (NO) is known as messenger molecule in cells and it is involved in many physiological and pathological functions[7-10]. NO is synthesized from L-arginine by nitric oxide synthase (NOS). NOS is divided into 3 major isoenzymes: neuronal NOS (nNOS), endothelial NOS (eNOS) and inducible NOS (iNOS). Excessive NO release by iNOS is known to cause cellular toxicity and pro-inflammatory condition[10,11]. It was reported that ischemia-reperfusion injury regulates NOS expression in rat sciatic nerve, suggesting that NO plays a potential role in peripheral nerve injury[12,13].

Neurotrophins play critical roles in the develo-pment of nervous system and synaptic plasticity [14,15]. They also protect neurons from degeneration, promote regeneration of injured nerves and enhance differentiation of neural stem cells by activating tyrosine kinase receptors (trk) and its down-stream signal pathways[16-18]. Therefore, it has been suggested that neurotrophins might be used as a potential therapeutic drug for the disorders of nervous system [19]. Of these, brain-derived neurotrophic factor (BDNF) is a unique neurotrophin and it is synthesized in neurons, transsynaptically transferred from neuron to neuron until reaching the target point[20-23].

C-Fos is the product of the immediate early genes and is rapidly expressed in the neurons in response to various stimuli. c-Fos expression is recognized as a marker of increased neuronal activity[24]. Because c-Fos expression is associated with stimuli-induced responses, it has been used for brain mapping and in searching for neural pathways[25-27]. In the mamm-alian brain, there are some specific areas receiving pain transmission. The area of periaqueductal gray (PAG), particular ventrolateral PAG (vlPAG), of the midbrain is an important brain region for pain perception[28]. Activation of neurons in the PAG indicates pain conduction from sciatic nerve injury[3].

Herbs are a major component of traditional medicine worldwide. Preparations of *Aconiti ciliare tuber(Aconitumcarmichaeli)* are employed in Chinese, Japanese and Korean traditional medicine[29]. *Aconiti ciliare tuber(Aconitumcarmichaeli)*has been found to be helpful in cases of acute rheumatism, arthritis, gout, neuralgia, and sciatica and has also been used for analgesic, antirheumatic, and neurological indi-cations[30]. Its complications are that it contains the highly toxic C_{19} diterpenoid alkaloids of aconitine, mesaconitine and hypaconitine[31]. After ingestion, patients sometimes complain of symptoms typical of aconitine poisoning. Death, rarely, may occur from ventricular arrhythmias, which is most likely devel-oped within the first 24 h[32,33]. *Aconiti ciliare tuber* are subjected to a process of "curing" which in this case involves steaming or boiling in water to reduce the toxicity by converting aconitine alkaloids to aconines and benzoylaconitines[34]. In the usage of this herb, it was reported that *Aconiti ciliare tuber* promotes blood circulation, strengthens the immune system, invigorates and retards the aging process and this herb has been used for the treatment of congestive heart failure, neuralgia, rheumatism, poor metabolism, sciatica, gout, etc.[35-37].

The effect of *Aconiti ciliare tuber* against neuro-pathic pain induced by sciatic crushed nerve injury has not been clarified. In the present study, we have prepared the aqueous extract of the *Aconiti ciliare tuber* and investigated the recovery rate of locomotor function, the expression of BDNF and iNOS proteins in injured sciatic nerves and the expressions of c-Fos in the PAG region following sciatic crushed nerve injury in rats.

Materials and methods

1. Experimental animals

Male Sprague-Dawley rats weighing 200 ± 10 g (6 weeks of age) were used. The experimental procedures were performed in accordance with the

animal care guidelines of National Institutes of Health (NIH) and the Korean Academy of Medical Sciences.

Theanimalswerehousedatacontrolledtemperature(20± 2℃) and maintained under light-dark cycles, each consisting of 12 h of light and 12 h of darkness (lights on from 07:00to19:00h), with food and water made available *ad libitum*. The rats were randomly divided into six groups(n=10 in each group): the normal group, the sham operation group, the operation(sciatic crushed nerve injury) group, the operation and 50mg/kg *Aconiti ciliare tuber*-treated group, the operation and 100mg/kg *Aconiti ciliare tuber*-treated group and the operation and 200mg/kg *Aconiti ciliare tuber*-treated group. The rats in the *Aconiti ciliare tuber*-treated groups orally received the aqueous extract of *Aconiti ciliare tuber* at the respective dose once a day from 1st day to 15th day at the commencement of the experiment and those in the control groups received an equivalent amount of saline for the same duration of time.

2. Aqueous extraction of *Aconiti ciliare tuber*

To reduce toxicity of *Aconiti ciliare tuber* by converting aconitine alkaloids to aconines and benz-oylaconitines, 600 g of the *Aconiti ciliare tuber*, 30g of the Glycyrrhizae radix and 60g of the Glycine max merr were boiled with 3,000ml of water for 4h. Then *Aconiti ciliare tuber* was extracted and dried[38]. To obtain the aqueous extracts of *Aconiti ciliare tuber*, 100g of the processed *Aconiti ciliare tuber* was washed, added to distilled water, heat-extracted, pressure-filtered, concentrated with rotary evaporator and lyophilized(EYELA, Tokyo, Japan). The result ing powder, weighing 20.98g(a yield of 21.98%), was diluted with distilled water to the concentrations needed.

3. Surgical procedure

To induce crush injury on the sciatic nerve in rats, a surgical procedure based on the previously described method was performed[39]. In brief, the right sciatic nerve was exposed through splitting incision on the gluteal muscle under pentobarbital anesthesia(50mg/kg,i.p.; Sigma Chemical Co., St.Louis, MO, USA). The sciatic nerve was carefully exposed and crushed for 30 sec using a surgical clip between the sciatic notch and the point of trifurcation. Subsequently, the surgical wound was sutured and recovered. In the sham operation rats, the sciatic nerve was exposed but crushing pressure on the nerve was not applied.

4. Walking track analysis

Functional recovery of the rats after sciatic crushed nerve injury was analyzed using a walking track assessment, which can be quantified by SFI. Exam-ination of the walking pattern was performed at one day intervals through the course of the experiment as in the previously described method[6]. Footprints were recorded in a wooden walking alley (8.2 × 42 cm) with a darkened goal box at the end. The floor of the alley was covered with white paper. The anatomical landmarks on the hind feet of the rats were smeared with finger paint. The rats were allowed to walk down the track, leaving their footprints on the paper. From the footprints, the following param-eters were calculated: distance from the heel to the top of the third toe (print length; PL), distance between the first and the fifth toe (toe spread; TS) and distance from the second to the fourth toe (intermediary toe spread; ITS). These parameters were taken both from the intact left (non-operated) foot (NPL, NTS and NITS) and from the injured right (experimental) foot (EPL, ETS and EITS). SFI values were obtained using following equation: (i) print length factor (PLF) = (EPL - NPL)/NPL; (ii) toe spread factor (TSF) = (ETS - NTS)/NTS; (iii) intermediary toe spread factor (ITSF) = (EITS - NITS)/NITS. These factors were then incorporated into the sciatic function index-formula: SFI = - 38.3 × PLF + 109.5 × TSF + 13.3 × ITSF (Fig. 1).

Interpolating identical values of PL, TS and ITS from the right and the left hind feet are close to
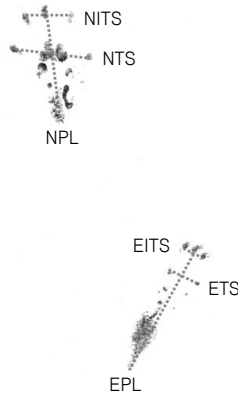
Fig. 1. Footprint of walking track analysis. After sciatic crushed nerve injury in rats, paired parameters of the print length (PL), toe spread (TS) and intermediary toe spread (ITS) were calculated. (E) Experimental side, (N) normal side, (EPL) experimental print length, (NPL) normal print length, (ETS) experimental toe spread, (NTS) normal toe spread, (EITS) experimental intermediary toe spread, (NITS) normal intermediary toe spread.

zero in normal rats. A value of -100 indicates complete impairment of walking ability.

5. Tissue preparation

At 15th day from surgery, the rats were weighed and overdosed with Zoletil 50®(10mg/kg,i.p.;Vibac Laboratories, Carros, France) following last walking track analysis. After a complete lack of response was observed, sciatic nerves were extracted from half of the rats for Western blot analysis, the other rats were transcardially perfused with 4% paraform-aldehyde(PFA) in 100 mM phosphate buffer(PB, pH7.4). The brains were dissected, post fixed in the same fixative overnight and transferred into a 30% sucrose solution for cryoprotection. Serial coronal sections of 40 μm thickness were made using a freezing microtome (Leica, Nussloch, Germany).

6. c-Fos immunohistochemistry

For immunolabeling of c-Fos in the vlPAG of each brain, c-Fos immunohistochemistry was performed. Free-floating tissue sections were incubated overnight with rabbit anti-Fos antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) at a dilution of 1:1000 and the sections were then incubated for 1 h with biotinylated anti-rabbit secondary antibody

(Vector Laboratories, Burlingame, CA, USA). The sections were subsequently incubated with avidin-biotin-peroxidase complex (Vector Laboratories) for 1 h at room temperature. Immunoreactivity was visualized by incubating the sections in a solution consisting of 0.05% 3,3-diaminobenzidine (DAB) and 0.01% H_2O_2 in 50 mM Tris-buffer (pH7.6) for approximately 3 min. The sections were then washed three times with PBS and mounted onto gelatin-coated slides. The slides were air-dried overnight at room temperature and coverslips were mounted using Permount®. As the negative control, the brain sections were like wise processed using normal goat serum in place of the primary antibody: no c-Fos-like immunoreactivity was observed.

7. Western blot analysis

Sciatic nerves were homogenized with an ice-cold whole cell lysate buffer containing 50 mM HEPES (pH 7.5), 150 mM NaCl, 10% glycerol, 1% Triton X-100, 1.5 mM magnesium chloride hexahy-drate, 1 mM ethyleneglycol-bis-(b-aminoethyl ether)-N, N-tetraacetic acid (EGTA), 1 mM phenylmethy-lsulfonyl fluoride (PMSF), 2mg/ml leupeptin, 1 mg/ml pepstatin, 1 mM sodium ortho vanadate and 100 mM sodium fluoride and the mixture was

incubated 20 min at 4 °C. It was centrifuged as 14,000 rpm 15 min, followed by quick freezing of the supernatant. The protein concentration was measured using a Bio-Rad colorimetric protein assay kit (Bio-Rad, Hercules,CA,USA). Protein of 50 mg was separated on SDS-polyacrylamide gels and transferred onto a nitrocellulose membrane (Schleicher & Schuell GmbH, Dassel, Germany). Rabbit BDNF antibody (1:1000;Santa Cruz Biotech) and rabbit iNOS antibody (1:1000;Santa Cruz Biotech) were used as primary antibodies. Horseradish peroxidase-conjugatedanti-rabbitantibody(1:2000;Santa Cruz Biotech) for BDNF and iNOS were used as a secondary antibody. Band detection was performed using the Western blot luminol reagent.

8. Data analyses

The data are expressed as the mean ± standard error of the mean (S.E.M). For comparisons among the groups, one-way ANOVA and Duncan's post-hoc test were performed with $P<0.05$ as an indication of statistical significance.

Results

1. *Aconiti ciliare tuber* enhanced SFI following sciatic crushed nerve injury

We measured sciatic functional index (SFI) using a walking track analysis to assess recovery rate of motor after sciatic crushed nerve injury in rats. The mean SFI in each group was determined on the 1st, 3rd, 5th, 7th, 9th, 11th, 13th and 15th day after sciatic crushed nerve injury.

The SFI in the sham operation group was -7.09 ± 1.25 on the 1st day, -6.09 ± 1.51 on the 3rd day, -6.90 ± 1.59 on the 5th day, -8.00 ± 0.98 on the 7th day, -6.42 ± 0.93 on the 9th day, -6.09 ± 1.41 on the 11th day, -8.03 ± 1.19 on the 13th day and -5.99 ± 1.29 on the 15th day at the commencement of the experiment.

The SFI in the operation group was -100 on the 1st day, -89.14 ± 1.83 on the 3rd day, -88.65 ± 1.69 on the 5th day, -78.33 ± 3.18 on the 7th day, -74.66 ± 4.28 on the 9th day, -75.64 ± 2.70 on the 11th day, -68.21 ± 2.39 on the 13th day and -59.32 ± 7.50 on the 15th day at the commencement of the experiment.
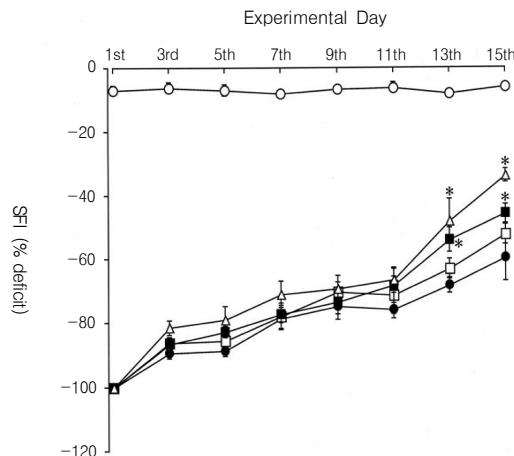
Fig. 2. Effect of Aconiti ciliare tuber on the sciatic functional index (SFI).

The values are represented as the mean ± S.E.M. * represents $P < 0.05$ compared to the operation (sciatic crushed nerve injury) group. (○) Sham operation group, (●) operation group, (□) operation and 50 mg/kg Aconiti ciliare tuber-treated group, (■) operation and 100 mg/kg Aconiti ciliare tuber-treated group, (△)operation and 200 mg/kg Aconiti ciliare tuber-treated group.

The SFI in the operation and 50 mg/kg *Aconiti ciliare tuber*-treated group was -100 on the 1st day, -86.23 ± 1.35 on the 3rd day, -85.47 ± 1.47 on the 5th day, -77.76 ± 4.10 on the 7th day, -70.34 ± 3.26 on the 9th day, -71.25 ± 5.91 on the 11th day, -63.03 ± 3.19 on the 13th day and -52.28 ± 3.14 on the 15th day at the commencement of the experiment.

The SFI in the operation and 100 mg/kg *Aconiti ciliare tuber*-treated group was -100 on the 1st day, -86.33 ± 1.91 on the 3rd day, -82.68 ± 1.76 on the 5th day, -77.06 ± 2.62 on the 7th day, -73.46 ± 3.78 on the 9th day, -68.29 ± 5.21 on the 11th day, -53.98 ± 3.83on the 13th day and -45.67 ± 2.96 on the 15th day at the commencement of the experiment.

The SFI in the operation and 200 mg/kg *Aconiti ciliare tuber*-treated group was -100 on the 1st day, -81.38 ± 2.22 on the 3rd day, -78.75 ± 4.14 on the 5th day, -71.00 ± 4.16 on the 7th day, -69.03 ± 4.03 on the 9th day, -66.43 ± 3.98on the 11th day, -47.97 ± 6.65 on the 13th day and -33.90 ± 2.16 on the 15th day at the commencement of the experiment.

In the present results, the SFI of the sham operation group continued near zero level during the experimental period. At the 1st day, the SFI in the all operation groups dropped to -100. In the operation group, the SFI value slowly increased during the experimental period. In the operation and *Aconiti*

ciliare tuber-treated groups, SFI value was enhanced from the 13th day and 200 mg/kg *Aconiti ciliare tuber* showed statistically significant recovery effect. On the 15th day from the commencement of the experiment, 100 mg/kg and 200 mg/kg *Aconiti ciliare tuber* showed statistically significant recovery effect. These results indicate that treatment with *Aconiti ciliare tuber* promoted functional locomotor recovery following sciatic crushed nerve injury in a dose-dependent manner (Fig. 2).

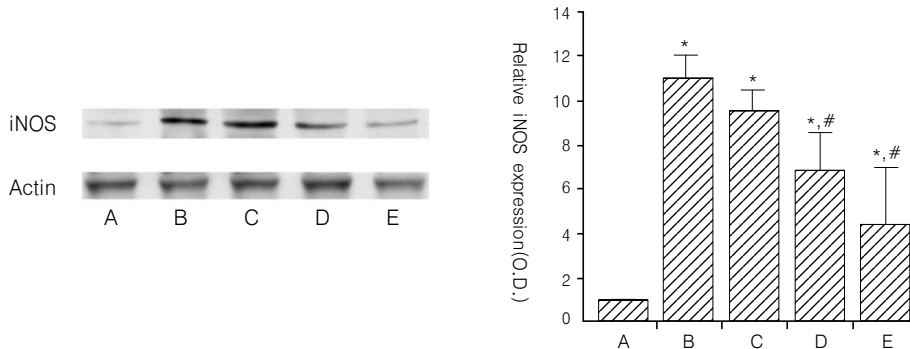## 2. Western blot analysis of BDNF expression

The expression of BDNF protein (14 kDa) in the sciatic nerve was increased by crushed nerve injury and treatment with *Aconiti ciliare tuber* enhanced BDNF protein as a dose-dependent manner. The expression level of BDNF in the sham operation group was set as 1.00. The expression of BDNF in the operation group was 1.79 ± 0.03, 2.01 ± 0.17 in the operation and 50 mg/kg *Aconiti ciliare tuber*-treated group, 2.40 ± 0.28 in the operation and 100 mg/kg *Aconiti ciliare tuber*-treated group and 2.92 ± 0.22 in the operation and 200 mg/kg *Aconiti ciliare tuber*-treated group, respectively (Fig. 3).

These results indicate that sciatic crushed nerve injury increased expressions of BDNF protein in the sciatic nerve.
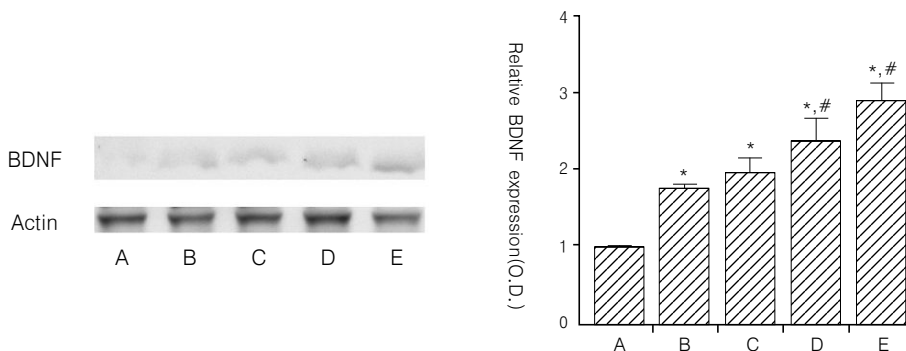
Fig. 3. Effect of Aconiti ciliare tuber on the relative BDNF protein expression in sciatic nerve.
The values are represented as the mean ± S.E.M. * represents $P < 0.05$ compared to the sham operation group. # represents $P < 0.05$ compared to the operation group. (A) Sham operation group. (B) operation group. (C) operation and 50 mg/kg Aconiti ciliare tuber-treated group. (D) operation and 100 mg/kg Aconiti ciliare tuber-treated group, (E) operation and 200 mg/kg Aconiti ciliare tuber-treated group.

Fig. 4. Effect of Aconiti ciliare tuber on the relative iNOS protein expression in sciatic nerve.

The values are represented as the mean ± S.E.M. * represents $F < 0.05$ compared to the sham-operation group. # represents $F < 0.05$ compared to the operation group. (A) Sham operation group, (B) operation group, (C) operation and 50 mg/kg Aconiti ciliare tuber-treated group, (D) operation and 100 mg/kg Aconiti ciliare tuber-treated group, (E) operation and 200 mg/kg Aconiti ciliare tuber-treated group.

3. Western blot analysis of iNOS expression

The expression of iNOS protein (140 kDa) in the sciatic nerve was increased by crushed nerve injury and treatment with *Aconiti ciliare tuber* suppressed iNOS protein expression in a dose-dependent manner. The expression level of iNOS in the sham operation group was set as 1.00. The expression of iNOS in the operation group was 11.12 ± 1.03, 9.62 ± 0.90 in the operation and 50 mg/kg *Aconiti ciliare tuber*-treated group, 6.88 ± 1.73 in the operation and 100

mg/kg *Aconiti ciliare tuber*-treated group and 4.46 ± 2.55 in the operation and 200 mg/kg *Aconiti ciliare tuber*-treated group, respectively (Fig. 4).

These results indicate that sciatic crushed nerve injury increased expressions of NOS protein in the sciatic nerve.

4. Effect of *Aconiti ciliare tuber* on c-Fos expression

The number of c-Fos-positive cells in the vlPAG
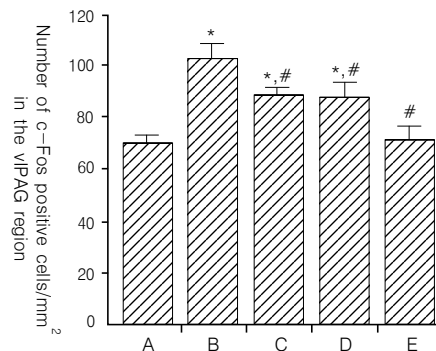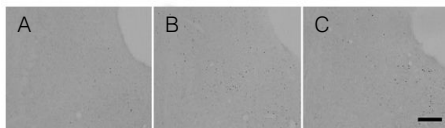
Fig. 5. Effect of Aconiti ciliare tuber on the c-Fos expression in ventrolateral periaqueductal gray (vlPAG).

Upper: Photographs of the c-Fos-positive cells. a, Sham operation group b, operation group c, operation and 200 mg/kg Aconiti ciliare tuber-treated group. The scale bar represents 200 µm.
Lower: Mean number of c-Fos-positive cells in each group. The values are represented as the mean ± S.E.M. * represents $F < 0.05$ compared to the sham operation group. # represents $F < 0.05$ compared to the operation group. (A) Sham operation group, (B) operation group, (C) operation and 50 mg/kg Aconiti ciliare tuber-treated group, (D) operation and 100 mg/kg Aconiti ciliare tuber-treated group, (E) operation and 200 mg/kg Aconiti ciliare tuber-treated group.

was $69.36 \pm 3.74/\text{mm}^2$ in the sham operation group, $103.33 \pm 5.63/\text{mm}^2$ in the operation group, $88.11 \pm 3.70/\text{mm}^2$ in the operation and 50 mg/kg *Aconiti ciliare tuber*-treated group, $88.00 \pm 5.24/\text{mm}^2$ in the operation and 100 mg/kg *Aconiti ciliare tuber*-treated group and $71.54 \pm 4.33/\text{mm}^2$ in the operation and 200 mg/kg *Aconiti ciliare tuber*-treated group.

In the present results, c-Fos expression in the vlPAG was increased by sciatic crushed nerve injury and treatment with *Aconiti ciliare tuber* significantly suppressed c-Fos expression in the vlPAG. *Aconiti ciliare tuber* at 200 mg/kg showed the most potent suppressing effect on c-Fos expression in the vlPAG (Fig. 5).

Discussion

Peripheral nerves systems are often damaged by crush, compression, ischemia and various diseases. In the sciatic crushed nerve injury, the injured limb displays hyperalgesia, pain-related gait and swelling[1]. This study researched that SFI level as the functional recovery, BDNF expression, iNOS expression and c-Fos expression after sciatic crushed nerve injury in rats and treated with aqueous extracts of *Aconiti ciliare tuber*.

The SFI derived from walking track analysis in rats provides a reliable and easily quantifiable method for the assessing of motor function after sciatic nerve injury[40]. This gait analysis is based on the fact that rats normally walk on their digits and metatarsal footpads. Print length is therefore short in normal animals. Sciatic nerve injury causes functional loss of both extensor muscles and flexor muscles of the foot, causing drop of foot. In sciatic crushed nerve injury model, Vogelaar *et al.*[41]. reported that although sensory and motor reinnervation of the paw were fully established at 3 weeks after nerve injury, persistent pain still existed and the animals could not support their weight on the injured paw. The rats subjected to crush injury sometimes walk by their dorsum of the affected foot or load their weight on the medial part of the affected foot.

These observations might be due to compensatory immobilization to painful dysesthesia as well as neurological loss. In the present study, right sciatic crushed nerve injury in rats resulted in the characteristic pattern of the footprints, representing reduction in the SFI value. The SFI value of the rats in the operation and *Aconiti ciliare tuber*-treated groups was sharply increased from 13th day of the experiment, whereas the SFI value of the rats in the operation group was slight increased until the 15th day of the experiment. The present results indicate that treatment with *Aconiti ciliare tuber* accelerated functional recovery from the locomotor deficit after sciatic crushed nerve injury.

BDNF has been suggested to play important roles in peripheral nerve regeneration, although the mechanism is still unclear. BDNF is also implicated in the development of neuropathic pain after peripheral nerve injury[42-45]. Increase of BDNF helps motor and sensory reinnervation[46] and Marcol *et al.*[47]. suggested that BDNF plays reinnervation in high concentrations and development of neuropathic pain or peripheral never injury in low concentrations. In the present results, BDNF protein expression in the sciatic nerve was increased by induction of crushed injury on sciatic nerve, meanwhile treatment with *Aconiti ciliare tuber* enhanced BDNF protein expression in the sciatic nerve following crushed nerve injury. There is argument that BDNF may promote development of neuropathic pain, however it is clear that increase of BDNF is helpful for the recovery of motor and sensory functions. As present results show, increased BDNF expression by administration of *Aconiti ciliare tuber* can promote nerve regeneration and reinnervation of the injured sciatic nerve.

NO exerts different actions according to the NOS isozymes. NO derived from eNOS and NO derived from nNOS promote repairment and suppress production of pro-inflammatory cytokines, while NO derived from iNOS induces inflammation and release production of pro-inflammatory cytokines[48]. NO produced by eNOS and nNOS continues within

48 h, while NO produced by iNOS continued until 14 days in the endoneurium after sciatic nerve crushed injury[48]. Yamada *et al.*[49] reported that plasma nitrite and nitrate (p-NOx) were significantly increased by *Aconiti ciliare tuber* administration in humans and they suggested that *Aconiti ciliare tuber* increased blood circulation by increase of eNOS. NO also contributes development of nociception and it may mediate some of the neuropathic pain syndromes following peripheral nerve injuries[2]. Hence, iNOS-originated NO is known to induce neuropathic pain. Shin *et al.*[50] reported that inhibition of iNOS promoted recovery of motor function of sciatic nerve following ischemia-reperfusion damage. In the present results, *Aconiti ciliare tuber* administration inhibited iNOS expression in crush injured sciatic nerve. The present results reveal that suppressed iNOS by *Aconiti ciliare tuber* might reduce hyperalgesia and inflammatory damage in sciatic crushed nerve injury.

The level of c-Fos in the frontal cortex, thalamus and PAG represent degree of pain perception. Narita *et al.*[3] showed that c-Fos expression in those areas was increased by sciatic nerve ligation in rats. Liou *et al.*[51] reported that *Aconiti ciliare tuber* had anti-nociceptive effect and might be associated with opioid μ-receptors in tail flick test. In the present results, c-Fos expression in vlPAG was significantly increased following crush injury on rat sciatic nerve. Administration of *Aconiti ciliare tuber* suppressed c-Fos expression in vlPAG. The present results indicate that *Aconiti ciliare tuber* inhibited crush injury-induced neuronal activation in the vlPAG, suggesting that *Aconiti ciliare tuber* has analgesic effect on nerve injury.

Conclusion

In the present study, we showed that *Aconiti ciliare tuber* increased SFI following sciatic crushed nerve injury in rats. The recovery mechanisms of SFI by *Aconiti ciliare tuber* might be ascribed to the increase of BDNF expression for nerve regene-ration and reinnervation and to the suppression of iNOS expression for inhibiting nerve inflammation. As the results, c-Fos expression in the vlPAG was decreased by treatment with *Aconiti ciliare tuber*, representing that *Aconiti ciliare tuber* exerted analg-esic effect on sciatic crushed nerve injury. Here in this study, we showed that *Aconiti ciliare tuber* can be used as a useful herb for peripheral nerve injury and can be used in a new therapeutic intervention for pain control and functional recovery from the peripheral nerve injury.

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