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Functional Recovery Enhancement Following Injury to Rodent Peroneal Nerve by Lion's Mane Mushroom, *Hericium erinaceus* (Bull.: Fr.) Pers. (Aphyllophoromycetideae)

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ABSTRACT: Peripheral nerve injury represents a huge burden to society. Following peripheral nerve injury, improved behavioral outcome may be the most important evidence of functionality of axonal regeneration after any repair strategy. Nerve-crush injury is a well-established axonotmetic model in experimental regeneration studies to investigate the impact of various pharmacological treatments. *Hericium erinaceus* is a temperate mushroom but is now being cultivated in tropical Malaysia. In this study, we investigated the activity of aqueous extract of *H. erinaceus* fresh fruitbodies in promoting functional recovery following an axonotmetic peroneal nerve injury in adult female Sprague-Dawley rats with a long-term view toward the possible use of this mushroom in the treatment of nerve injury. Functional recovery was assessed in the behavioral experiment by walking-track analysis and toe-spreading reflex. The peroneal functional index (PFI) was determined before surgery and after surgery, as the rats showed signs of recovery. Analysis of the PFI indicated that the return of hind-limb function occurred by day 10 to 14 and by day 14 to 17 in the treated and control (nontreated) groups, respectively. Normal toe-spreading in the crushed limb was achieved by day 7 to 10 and day 12 to 17 in the treated and control group, respectively. These results suggest that daily administration of aqueous extract of *H. erinaceus* fresh fruitbodies has a beneficial effect on the recovery of injured rat peroneal nerve in the early stages of regeneration. The PFI and toe-spreading reflex improved faster in the treated group than in the nontreated group.

KEY WORDS: *Hericium erinaceus*, functional recovery, peripheral nerve, crush injury, peroneal functional index, toe-spreading reflex, medicinal mushrooms

ABBREVIATIONS

E: experimental; **DHEA:** dehydroepiandrosterone; **EDL:** the extensor digitorum longus; **MnSOD:** manganese superoxide dismutase; **N:** normal; **NGF:** nerve growth factor; **PFI:** peroneal functional index; **PLF:** print length factor; **SFI:** sciatic functional index; **TFI:** tibial functional index; **TSF:** toe-spread factor.

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I. INTRODUCTION

Medicinal properties of *Hericium erinaceus* (Bull.: Fr.) Pers. (also known as Lion's Mane, Monkey's Head, Hedgehog Fungus, Satyr's Beard, Pom Pom Blanc, Igelstachelbart, and Yamabushitake), Hericiaceae, Aphyllophoromycetideae have been well-known for hundreds of years in traditional Chinese and Japanese cooking and herbal medicine to treat various human diseases, and hot aqueous extracts from dried fruitbodies are used as a health drink called "Houtou." The fruitbodies are composed of numerous constituents, such as polysaccharides,[1] proteins,[1] lectin,[2] and hericenones.[3–5]

A study carried out at the Third People's Hospital of Shanghai showed that *H. erinaceus*, in tablet form, was effective in treating ulcers, inflammations, and tumors of the alimentary canal.[6] The cytoprotective effect of *H. erinaceus* freeze-dried fruitbodies against ethanol-induced gastric mucosal injury in rats was investigated. The rats had less gastric-mucosal damage, decreased edema, and no submucosal leucocyte infiltration when compared to nontreated rats.[7]

The most promising activity of *H. erinaceus* is the stimulation of nerve growth factor (NGF) synthesis by hericenones from fruitbodies[3–5] and erinacines from mycelium.[8–13] An exopolysaccharide derived from *H. erinaceus* promotes neuronal differentiation and survival.[14] Neurotrophic activities derived from dried fruitbodies of *H. erinaceus* have also been studied in rat hippocampal slice neurons.[15,16] Our previous study has shown that aqueous extracts of this mushroom grown in a tropical environment could stimulate neurite outgrowth of the cultured cells of the neural hybrid clone NG108-15.[17] These findings prove that *H. erinaceus* may have the potential to stimulate neurons to regrow in the treatment of senility and Alzheimer's disease, and for repairing neurological trauma from strokes, improving muscle or motor response pathways, and increasing cognitive function.

Peripheral nerve problems are common and encompass a wide spectrum of traumatic injuries, diseases, tumors, and iatrogenic lesions. The incidence of traumatic injuries is estimated as more than 500,000 new patients annually.[18] Injuries to peripheral nerves result in partial or total loss of motor, sensory, and autonomic functions in the involved segments of the body. Reinnervation of denervated targets can be achieved by the regeneration of injured axons or by collateral branching of undamaged axons in the surrounding.[18]

Common types of experimentally induced injuries include crush injury that causes axonal interruption but preserves the connective sheaths (axonotmesis), complete transection disrupting the whole nerve trunk (neurotmesis), and resection of a nerve segment inducing a gap of certain length. Nerve-crush injury is a well-established model in experimental regeneration studies to investigate the impact of various pharmacological treatments.[18–24] It is known that after the injury due to the tissue destruction, free-oxygen radicals increase and cause tissue damage.[25–28]

Rodents, particularly the rat and mouse, have become the most frequently utilized animal models for the study of peripheral nerve regeneration because of the widespread availability of these animals as well as the distribution of their nerve trunks, which is similar to humans.[18,29] Gutmann and Guttmann[30] demonstrated that the loss of ability to spread the toes of the hind limb is a reliable parameter for the evaluation of the extent of injury to the sciatic nerve and for monitoring the recovery. However, the method proposed was quite rudimentary and did not allow the quantification of any parameter. A reliable and reproducible quantitative method for the assessment of functional condition, known as the sciatic function index (SFI), was introduced by De Medinaceli et al.[31] They designed a quantitative method of analyzing hindlimb performance by examining footprints based on several measurements of the footprints made on X-ray film. Carlton and Goldberg[32] introduced the tibial functional index (TFI) and the peroneal functional index (PFI), which were later modified by Bain et al.[33] The use of walking-track analysis has been widely used in the rat sciatic nerve studies and is considered as an assessment of global function recovery.[32–34]

Research on the medicinal value of *H. erinaceus* grown in Malaysia, a tropical country, is minimal and yet to be explored. To our knowledge, no information is available on the nerve regeneration and repair property of the locally grown mushroom

H. erinaceus. Therefore, the aim of this study was to assess the peroneal nerve regeneration activity of aqueous extract of *H. erinaceus* fresh fruitbodies in adult female Sprague-Dawley rats after crush injury. Functional recovery was assessed in behavioral experiments by walking-track analysis and toe-spreading reflex.

II. MATERIALS AND METHODS

A. Preparation of Aqueous Extracts and Animal Grouping

H. erinaceus fresh fruitbodies were obtained from a mushroom farm in Tanjung Sepat, Selangor, Malaysia. Fresh fruitbodies were boiled with distilled water at a ratio of 1:1 for 30 minutes with agitation, left covered for 30 minutes, cooled and filtered. The use of rats was approved by the Animal Care and Use Committee of the Faculty of Medicine, University of Malaya, Approval Number ANA/16/03/2007/MDKN(R). Twenty adult female Sprague-Dawley rats weighing 180–200 g were randomly assigned to two groups. The control group (n = 10) received daily oral administrations of distilled water (10 mL/kg body weight/day), and the treatment group (n = 10) received the aqueous extract of fresh fruitbodies (10 mL/kg body weight/day) for 14 days to function as pretreatment before surgery.

B. Surgical Procedure

On the 14th day, the rats were anesthetized with an intraperitoneal injection of 3.5% chloral hydrate (10 mL/kg body weight), then shaved and washed with antiseptic solution before positioning for surgery. The right sciatic nerve and its two major branches were exposed through a gluteal muscle-splitting incision. A reliable and reproducible crush injury was created using a fine watchmaker forceps no. 4 for 10 seconds on the peroneal nerve at 10 mm from the distal muscle, and complete crush was confirmed by the presence of a translucent band across the nerve (Fig. 1). The incision was then closed in layers (muscle and skin) with absorbable sutures. All operations were performed on the right limb, and the left limb served as an unoperated control. After closing the incision with sutures, veterinary wound powder was applied to the wounds. The extract or distilled water was continuously fed for another 20 days. All rats were observed for general well-being and had *ad libitum* access to food and water throughout the study.

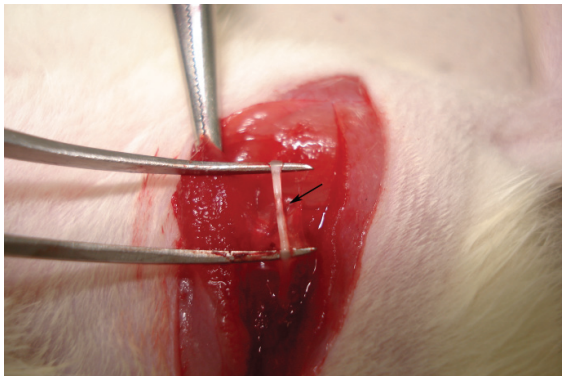


FIGURE 1. Complete crush of peroneal nerve is confirmed by the presence of a translucent band (as indicated by an arrow) across the nerve.

C. Functional Assessment of Limb Recovery

1. Walking-Track Analysis

The rats were allowed conditioning trials in a walking track (8.2 × 42 cm) darkened at one end. White office paper cut to the appropriate dimensions was placed on the bottom of the track. The rat's hind limbs were dipped in Chinese ink, and the rat was permitted to walk down the track, leaving its hind footprints on the paper (Fig. 2). Footprints were obtained before surgery (day 0) and on day 4, 7, 10, and 14 after surgery, as the rats showed signs of recovery.

Several trials are required to obtain the most representative prints for analysis.[33,34] In the beginning, they often stop to explore the corridor; thereafter, they walk steadily to the darkened cage.[31] The rat may stand up, putting all its weight onto its rear legs and creating untypically long print length (Fig. 3A). Some prints were unmeasurable due to smearing of the print, dragging of the tail

FIGURE 2. Walking-track apparatus. Rat in an 8.2 × 42 cm walking-track apparatus lined with white office paper. After the hind limbs of the rat are dipped in Chinese ink, the rat walks towards the darkened end of the corridor.

across the print, or contamination with front paw prints (Fig. 3B).

PFI is based on multiple linear regression analyses of factors derived from measurements of walking tracks in rats with peroneal nerve injury. The factors that contributed to PFI were print length factor (PLF) and toe-spread factor (TSF). Paired measurements of the print length (distance from heel to toe) (PL) and the toe-spread (distance from the first to fifth toes) (TS) were taken for the unoperated/normal (N) foot and the corresponding operated/experimental (E) foot.[33] A factor was generated from each of the measurements of the walking track by subtracting the normal from the experimental value and dividing this difference by the normal measurement:

$$\text{Print length factor (PLF)} = \frac{\text{EPL-NPL}}{\text{NPL}}$$

$$\text{Toe-spread factor (TSF)} = \frac{\text{ETS-NTS}}{\text{NTS}}$$

The multiple linear regression analysis performed between the peroneal nerve deficit and the factors from the walking tracks gave the equation for PFI.

$$\text{PFI} = 174.9\left(\frac{\text{EPL-NPL}}{\text{NPL}}\right) + 80.3\left(\frac{\text{ETS-NTS}}{\text{NTS}}\right) - 13.4$$

The PFI oscillates around 0 to –10 for normal nerve function, and around –100 for total dysfunction, such as would result from a complete transection of the sciatic nerve.[35] A complete recovery of function was determined when the PFI for each group plateaued or returned to its presurgery value.

2. Toe-Spreading Reflex

The rats were inspected everyday after surgery. During these inspections, each rat was held by its tail above a surface and lowered towards it and carefully observed for a minute or two.[36] Activities were classified according to the toe-spreading reflex of the affected right hind limb: 0, no spreading; 1, minimal spreading; 2, average spreading; 3, normal spreading. The rate of peroneal nerve regeneration was calculated by dividing the distance of the crushing site from the distal muscle (10 mm) by the day normal spreading is achieved.

D. Statistical Analysis

The means of the data were subjected to a one-way analysis of variance (ANOVA) and the significance of the difference between the means was determined by the Duncan's multiple-range tests at 95% least-significant difference ($p < 0.05$).

III. RESULTS AND DISCUSSION

No rats in the two groups showed any sign of infection or foot ulceration at any time throughout

the experiment. Normal gait was recorded as the hind-limb toes fully spread in each group before surgery. Crush injury to the peroneal nerve results in paralysis of the extensor digitorum longus (EDL) muscle. Flexion contracture ("drop foot"), as shown in Figure 4, was observed due to the lack of dorsal flexion of the ankle. The rats tend to drag the dorsum of their foot until reinnervation of the EDL muscle.

The clinically relevant outcome is end-organ functional recovery, which is the ultimate test of nerve regeneration.[37] Functional evaluation showed that recovery in the treated group began on day 4, whereas the crushed limb in the control group remained dysfunctional. Rats in the control group showed clumping of toes and dragging of the injured foot (Fig. 5A). These rats are recorded as having unmeasurable walking tracks. On the other hand, the treated group demonstrated toe-spreading and clear footprints on the walking tracks (Fig. 5B).

Analysis of PFI, as shown in Table 1, indicated that the return of hind-limb function occurred by 14 or 17 days after surgery in 5 rats each of control group. Rats treated with aqueous extract of fresh fruitbodies experienced return of function by 10 and 14 days after surgery in 8 rats and 2 rats, respectively. When the group's mean PFI were compared at each time interval, the mean PFI of the treated

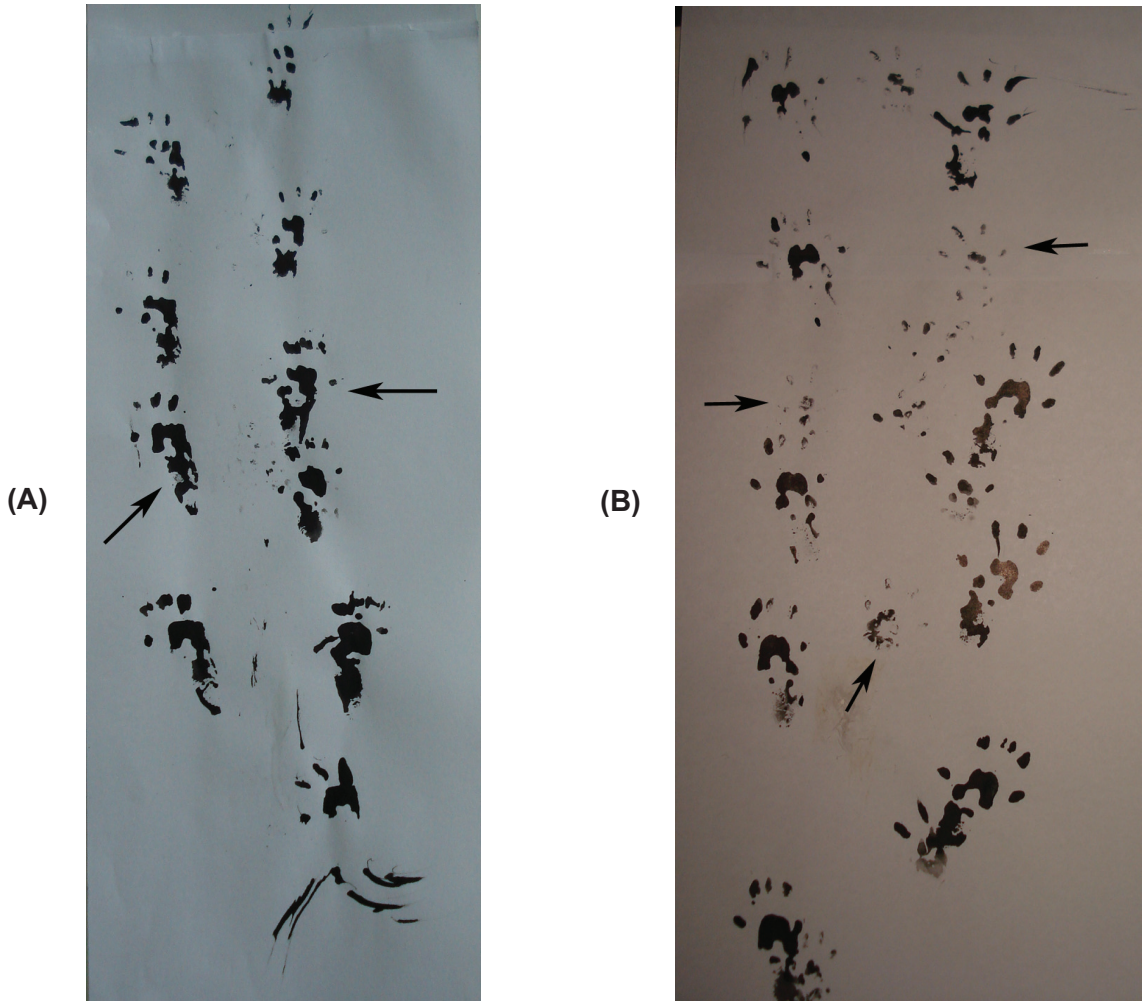


FIGURE 3. Unmeasurable walking tracks. (**A**) Long print length as indicated by arrows when a rat stands up and puts all its weight onto its hind limbs. (**B**) Contamination with front paw prints as indicated by arrows.

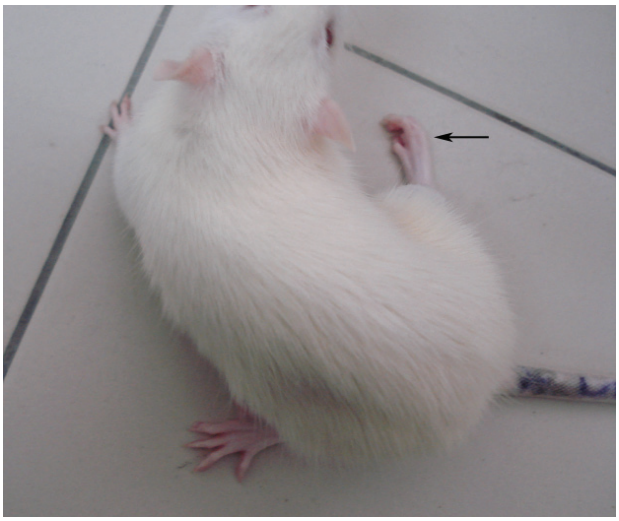
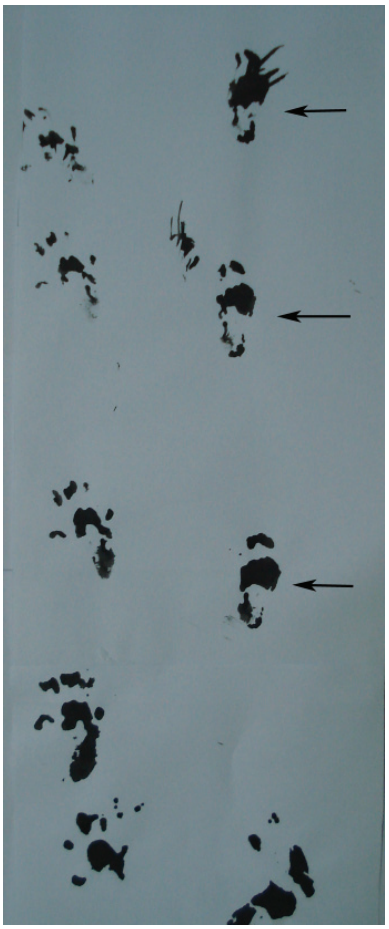


FIGURE 4. Gait changes associated with peroneal nerve injury–joint contracture, making measurement impossible because the rat walks on the dorsum of the affected foot. Arrow indicates the operated limb.



(Fig. 5Ai)

(Fig. 5Aii)

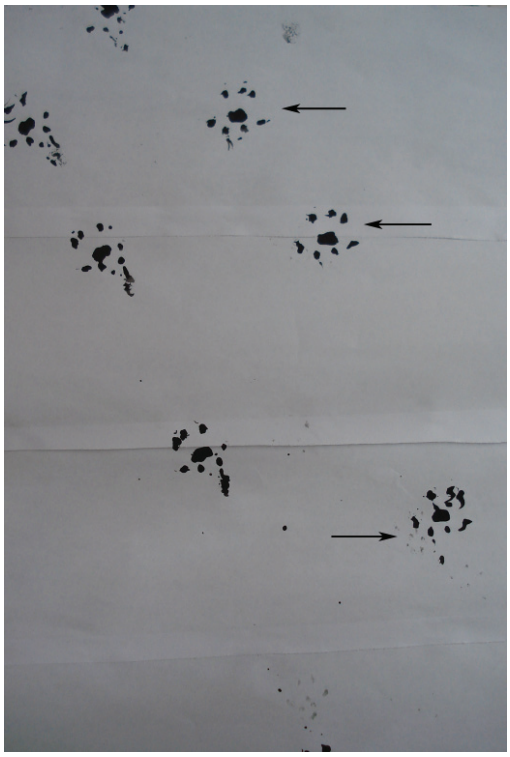
rats was significantly less than the control group on day 0, 7, 10, and 14 ($p < 0.05$). Print length is shorter at first[33] and will increase back to normal with time or as functional recovery takes place.

With regard to the toe-spreading reflex (Table 2), normal spreading appeared between 12 (1 rat), 13 (2 rats), 15 (2 rats), and 17 days (5 rats) after surgery in the control group. However, in the treated group, the deficit completely disappeared 7 (3 rats), 9 (2 rats), and 10 days (5 rats) after surgery. The rate of peroneal nerve regeneration was almost twofold higher in the treated group compared to the control group ($p < 0.05$). Figure 6 shows minimal toe-spreading on the right limb in the control group and normal toe-spreading in the treated group 7 days after surgery. Thus, the treated rats showed a faster recovery of the toe-spreading reflex when compared to the controls.

Gait and walking-track analysis have been used clinically, both to describe lower limb nerve deficits and to assess function. The hind-limb function served by the sciatic nerve and its branches in the rat can be quantitatively, reliably, and easily assessed by gait analysis through footprints.[33] This method is simple to perform, with minimal discomfort to the rat.[38] Furthermore, walking-track analysis is a direct measurement of function, whereas electrophysiologic and morphometric histologic analyses are indirect methods that may not necessarily correlate with functional outcome.[39]

The recovery of a muscle is a very complex and prolonged process. It sets in with the innerva-

(Fig. 5Bi)



(Fig. 5Bii)

FIGURE 5. Walking tracks of footprints after 4 days of right peroneal nerve-crush injury. Arrows indicate footprints of the operated limb. (**Ai**) and (**Aii**) Footprints in control group—distilled water (10 mL/kg body weight per day). The palsy after interruption of the peroneal nerve is characterized by flexion contracture of the paws ("drop foot"), absence of toe-spreading reflex, and some dragging of the operated limb. (**Bi**) and (**Bii**) Footprints in treatment group—aqueous extract of *Hericium erinaceus* fresh fruitbodies (10 mL/kg body weight per day). Toe-spreading and clear footprints of the operated limb are demonstrated on the walking tracks.

TABLE 1
Return of Function Following Crush Injury to the Peroneal Nerve as Shown by Peroneal Functional Index (PFI). PFI of Rats Treated with Aqueous Extract of *Hericium erinaceus* Fresh Fruitbodies Returned to Presurgery Values 4 to 7 Days Earlier Than Controls

	PFI values					
Group	**Day 0**	**Day 4**	**Day 7**	**Day 10**	**Day 14**	**Day 17**
Control	–15.63 ± 4.21[a]	Unmeasurable due to dragging of operated foot	–50.36 ± 7.13[a]	–32.71 ± 5.27[a]	–21.52 ± 8.88[a]	–18.88 ± 6.14
Treatment	–10.01 ± 3.40[b]	–52.88 ± 12.34	–21.44 ± 5.56[b]	–11.30 ± 4.49[b]	–10.71 ± 2.43[b]	

Note: Values on day 0 are before surgery. Data are expressed as means ± standard deviation (n = 10 for day 0, 7, and 10 in both groups, day 4 in treatment group, day 14 in control group; n = 2 for day 14 in treatment group; n = 5 for day 17 in control group). Means with different letters in the same column are significantly different ($p < 0.05$, one-way analysis of variance/ANOVA).

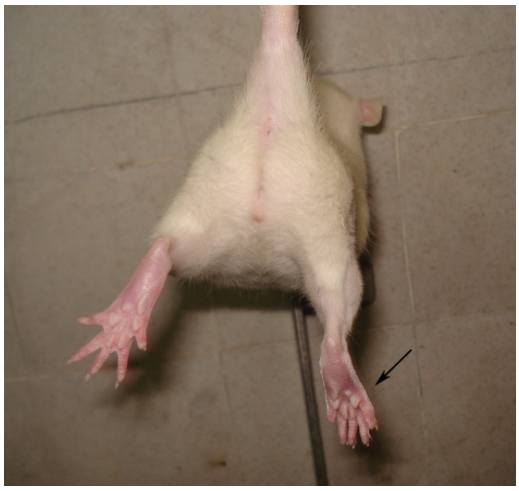
tion of the muscle by the first regenerating fibers. This stage is a necessary preliminary to recovery, but must not be identified with the recovery of the muscle as a functioning entity.[36] The process of recovery is not complete with the first return of a movement. The degree of movement, indicated by the toe-spreading reflex in the experiment described, increases for some time. Even after the full extent of movement has returned, the regeneration is still incomplete because the muscle continues for some weeks to increase in weight, the rate of increase presumably depending on the amount of use of the limb. Moreover, the nerve fibers themselves continue to increase in diameter for many months after functional recovery is apparently complete.[36] Recovery proceeds more slowly with greater distance between lesion and end organ.

Peripheral nerves may be subjected to crush injuries in a variety of circumstances, including motor vehicle accidents, fractures, dislocations, and natural disasters such as earthquakes.[40] In crush injury or second-degree Sunderland injury, there is an interruption of the nerve's axons, with subsequent Wallerian degeneration distal to the site of injury. The degenerative products are eliminated by the cooperative action of denervated Schwann cells and infiltrating macrophages. After this type of injury, the continuity of the endoneurial sheath is preserved, providing the necessary guidance for regenerating axons from the proximal nerve stump to their peripheral targets.[41,42] Axonal regeneration requires an adequate substrate of trophic and tropic factors, provided by reactive Schwann cells, macrophages, and the extracellular matrix within

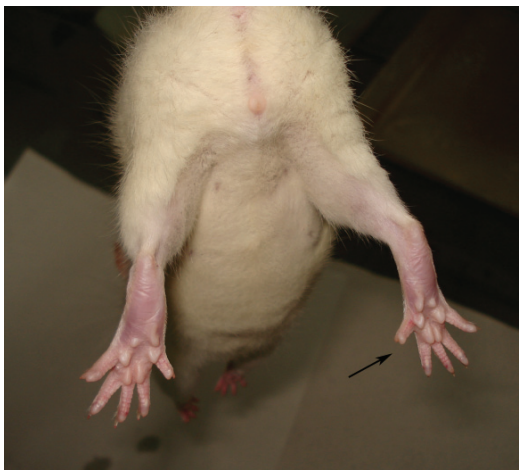
TABLE 2
Recovery of Toe-Spreading Following Crush Injury to the Peroneal Nerve. Toe-Spreading of Rats Treated with Aqueous Extract of *Hericium erinaceus* Fresh Fruitbodies Returned to Normal Spreading 5 to 10 Days Earlier than Controls

	Number of rats with normal spreading											Rate of peroneal nerve regeneration (mm/day)
Group	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	Day 17	
Control	0	0	0	0	0	1	3	3	5	5	10	0.66 ± 0.09[a]
Treatment	3	3	5	10	10	10	10	10	10	10	10	1.15 ± 0.20[b]

Note: n = 10 for both groups. Means with different letters in the last column are significantly different ($p < 0.05$, one-way analysis of variance/ANOVA).



(A)



(B)

FIGURE 6. Recovery of toe-spreading reflex after 7 days of right peroneal nerve-crush injury. Arrows indicate the operated limb. (**A**) Minimal toe-spreading on right limb in control group—distilled water (10 mL/kg body weight per day). (**B**) Normal toe-spreading on right limb in treated group—aqueous extract of *Hericium erinaceus* fresh fruitbodies (10 mL/kg body weight per day).

the degenerated nerve. From a clinical perspective, in second-degree Sunderland lesions, function will be almost completely restored, although the process requires many months.[43]

Functional deterioration following crush injury is not only related to the impact of the crush itself but also includes other important components, such as ischemia of the limb. Li et al.[44] suggested that the treatment of deferoxamine, an antioxidant, reduces ischemia or reperfusion injury after nerve compression. They hypothesized that therapeutic intervention with antioxidants protects the nerve from ischemia or reperfusion injury and yields a quick recovery from peripheral nerve compression injury. Studies on crush-injury models in peripheral nerves have shown better functional recovery when therapies were directed against ischemia-reperfusion injury by using antioxidants, lipid peroxidation inhibitors, and anti-inflammatory agents.[24,44]

The antioxidant and free-radical scavenging properties of mushrooms have been reported. Possible protective roles of antioxidant and free-radical scavenging properties of mushrooms are due to their ability to capture metals, inhibit lipoxygenase, and scavenge-free radicals.[45,46] *H. erinaceus*, a temperate mushroom, is now successfully grown in tropical conditions and has been reported to possess antioxidant activity.[47]

With regard to its assumed mechanism of action, aqueous extract of *H. erinaceus* fresh fruitbodies may act directly or indirectly by modifying the action of neurotrophic factors. Neurotrophic factors or regeneration-promoting factors have been suggested to play an essential role in the outcome of degeneration and regeneration processes in the peripheral nervous system, both to ensure proper innervation of the target tissues and to improve remyelination.[48] The improved regeneration observed after aqueous extract treatment may be related either to direct neurotrophic factor-like activity or to the promotion of the effects of nerve-derived neurotrophic factors.

Anti-inflammatory drugs such as methylprednisone, pregnenolone, and indomethacin have shown both experimental and clinical enhancement of neural functional recovery following acute spinal cord injuries.[49] Gudemez et al.[19] studied the application of dehydroepiandrosterone (DHEA), a weak androgenic steroid, immediately after crush injury. DHEA was shown to improve rat sciatic nerve regeneration and prevent the development of a "secondary" injury related to reperfusion. The

study was confirmed by the improved gait pattern of DHEA-treated rats, as measured by SFI, which returned to normal values faster than the control group. Lymphotoxin or tumor necrosis factor-β is a glycoprotein produced by activated T and B lymphocytes. It can enhance motor-function recovery of the crushed sciatic nerve in the early stages of regeneration. The mechanism of protection could be related to its ability to induce the synthesis of manganese superoxide dismutase (MnSOD), a mitochondrial enzyme involved in detoxification of superoxide radicals.[24] On the other hand, Al-Bishri et al.[50] also showed that the preoperative administration of the steroid bethamethasone improved the functional recovery of the rat sciatic nerve, as measured by the toe-spreading ability.

By taking natural products into consideration, the repair effect of the traditional Chinese medicinal herb *Achyranthes bidentata* root aqueous extract on the regeneration of the crushed common peroneal nerve in rabbits was studied by Ding et al.[51] by using a combination of electrophysiological assessment and histological investigation. The root extract could accelerate peripheral nerve regeneration in a dose-dependent manner.

Data obtained from this study suggest that daily treatment with the aqueous extract of *H. erinaceus* fresh fruitbodies provides a quicker recovery of function than no treatment at all. Because the extract administration increases the rate of recovery from peripheral nerve injury, patients who receive *H. erinaceus* may experience a more expeditious improvement in their quality of life and more complete functional recovery after injury.

IV. CONCLUSIONS

After peroneal nerve-crush injury, functional recovery was enhanced in rats treated with aqueous extract of *H. erinaceus* fresh fruitbodies, as assessed in behavioral experiments by walking-track analysis and toe-spreading reflex. Therefore, *H. erinaceus* could be a good candidate in facilitating functional recovery after peripheral nerve injury. However, prior to the application of the mushroom by the nutraceutical industry, the identification of the active compounds and the mechanisms by which these may treat or protect against nerve injury are highly warranted.

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