

Full Length Research Paper

The effect of *Lycium barbarum* polysaccharides on erectile function recovery in a rat model of cavernous nerve injury

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This study aimed to investigate the effect of *Lycium barbarum* polysaccharides (LBP) on erectile function recovery in a rat model of cavernous nerves (CN) injury. Adult male Sprague-Dawley rats were randomly divided into 3 groups: ten rats underwent sham operation (sham group), ten underwent bilateral CN crush injury (injured control group) and the other ten underwent bilateral CN crush injury with an oral administration of LBP for 12 weeks (LBP application group). Oxidative stress was evaluated by malondialdehyde (MDA) level, super oxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities in serum. Erectile function was assessed by CN electro-stimulation and CN regeneration was evaluated by toluidine blue staining at 12 weeks. After 12 weeks, the peak intracavernous pressure (ICP) and peak ICP per mean arterial pressure in the LBP application group were significantly higher than those in the injured control group, although lower than the sham group. The number of myelinated axons of CN in the LBP application group was more than that in the injured control group but fewer than the sham group. The results demonstrate that the application of LBP after CN crush injury promotes nerve regeneration and erectile function recovery.

Key words: Erectile dysfunction, *Lycium barbarum* polysaccharides, oxidative stress, nerve regeneration.

INTRODUCTION

Erectile dysfunction is still a major postoperative complication of radical prostatectomy, despite the developments of radical prostatectomy. The primary cause of erectile dysfunction is that the cavernous nerves (CN) may have been damaged by manipulation during the procedure (Woo et al., 2011). The recovery of erectile function is protracted, usually taking months to years, because it depends on regeneration of nerves from the remaining neural tissue. Recent studies reported that oxidative stress plays an important role on regeneration of injured nerve (Jovanović and Jovanović, 2011). Oxidative stress, defined as an imbalance of pro-

oxidants and antioxidants, can aggravate acute nerve injury due to the associated damage to cellular lipids, proteins and DNA, can cause a decline in cellular function (Fischer and Glass, 2010). Thus, alleviating oxidative stress may be an effective way of improving CN regeneration.

Lycium barbarum polysaccharides (LBP) isolated from *L. barbarum*, a traditional Chinese herbal medicine, have been identified as one of the active elements responsible for the biological activities. Owing to their antioxidant properties, LBP could be applicable in the treatment of trauma-induced oxidative stress. Recent studies have

shown that LBP can increase antioxidant efficacy and reduce neuronal damage (Li et al., 2011). Therefore, the aim of this research was to determine whether LBP is effective on erectile function recovery after CN injury.

MATERIALS AND METHODS

Plant material and extraction procedures

The dried fruits of *L. barbarum* originated from China were purchased from Wuhan city herb market (Hubei, China), and identified by Professor Luo Qiong, School of Medicine, Wuhan University. LBP were prepared as follows: briefly, 100 g of dried fruit samples were ground to powder and decocted in the boiling water for 2 h. Let the decoction rest for 2 h at room temperature. Then the decoction was freeze-dehydrated to obtain crude polysaccharides. To remove lipids, the crude polysaccharides were refluxed with 300 ml of chloroform: methanol solvent (2:1) (volume per volume). The residue was air-dried. The product was extracted in 300 ml of boiling water and then filtered. The filtrate was precipitated using 200 ml of 95% alcohol, dehydrated alcohol, and acetone, respectively. After filtering, the precipitate was collected and vacuum-dried, giving the resulting product LBP.

Experimental animals

A total of 30 healthy male Sprague-Dawley rats (3 months old, 250 - 300 g) were randomly divided into three equal groups. Ten animals underwent sham operation (sham group), composed of a midline incision and identification of the bilateral CN, with no further surgical intervention; ten underwent bilateral CN dissection and a subsequent crush injury using a hemostatic clamp (injured control group); and ten underwent bilateral CN crush injury as group 2, but followed by an oral administration of LBP for 12 weeks (LBP application group). All rats were obtained from the Experimental Animal Centre of Wuhan University (Wuhan, China), and the Institutional Animal Care and Use Committee of Wuhan University approved all the experimental protocols followed in this study.

Surgical procedures

Sprague-Dawley rats were anaesthetized with an intraperitoneal injection of sodium pentobarbital (40 mg/kg). The animals were maintained isothermic by placing them on a heated pad at 37°C. Through a low-midline abdominal incision, the bladder and prostate was exposed. With the aid of an operation microscope (Shanghai Medical Instruments Co. Ltd, Shanghai, China), the major pelvic ganglion were identified bilaterally at the area lateral to the prostate. From the major pelvic ganglion, bilateral CN were exposed to travel laterally into the corpus cavernosum. There was no additional surgical intervention in group 1. In group 2, bilateral CN were isolated and crush injury was applied. Specifically, the tips of a surgical needle driver (Cheng-He Microsurgical Instruments Factory, Ningbo, China) were positioned at a 90-degree angle and the CN was crushed at a constant 1 click pressure for 2 min per side. In group 3, after bilateral CN crushing as group 2, a post-operation oral administration of LBP (200 mg/kg body weight/day) was applied for 12 weeks. In all rats, the abdomen wall was sutured in 2 layers.

Oxidative stress evaluation

Oxidative stress was evaluated by malondialdehyde (MDA) levels,

superoxide dismutase (SOD) activity and glutathione peroxidase (GSH-Px) activity in serum at 1, 2, 4, 8 and 12 weeks. These above oxidative stress parameters were determined by respective assay kits (Nanjing Jiancheng Biology Research Institute, Nanjing, China) with the methods provided by the manufacturer.

Erectile responses assessment

The erectile response was assessed in all rats at 12 weeks by measuring peak intracavernous pressure (ICP) upon direct CN electro-stimulation. The bilateral CN were again exposed and isolated through a repeat midline abdominal approach. The skin overlying the penis was incised. Following ischiocavernous muscle dissection, the crura of penis were exposed. A 23 gauge scalp-vein needle filled with 250 U/ml of heparin solution was inserted into the right crus of penis and connected to polyethylene-50 tubing to measure the ICP. Systemic mean arterial pressure (MAP) was measured by inserting a 22 gauge scalp-vein needle into the left carotid artery via the side of the incised neck. A bipolar stainless steel electrode (2 mm diameter probes and separated by 1 mm) was used to directly stimulate the CN. Monophasic rectangular pulses were created by a computer with a steady current amplifier. The stimulus parameters were amplitude 1.5 mA, frequency 20 Hz, pulse width 0.2 milliseconds and duration 50 s. The ICP and MAP were recorded by Bioinformation Acquisition System (Chengdu TME Technology Co. Ltd, Chengdu, China). The mean of the peak right and left ICPs was then determined in each rat. Finally, the ratios of mean peak ICP/MAP in the three groups were calculated.

CN regeneration assessment

After functional testing, approximately 3 mm nerve tissue was harvested from the trunk of bilateral CN, distal to the site of injury, for toluidine blue staining. All harvested samples were fixed in 3% cold glutaraldehyde for one day. Then the samples were dehydrated with dehydrated alcohol and fixed with 1% osmium tetroxide. After being infiltrated with a graded araldite-propylene oxide mixture, the samples were embedded in epoxy resins. An LKB III Ultramicrotome (LKB Produkter A.B., Bromma, Sweden) was used for 1 µM ultrathin section of the embedded samples. Then the ultrathin section were stained with 1% toluidine blue and observed under light microscope immediately. Images were taken using an Olympus camera and analyzed by Image Pro-Plus Software, Version 6.0 (Media Cybernetics, Bethesda, MD, USA). The CN regeneration was evaluated by the number of myelinated axons of CN.

Statistical analyses

All data were expressed as mean ± standard deviation. Differences between groups were assessed by one-way analysis of variance and Student's t-test. Differences were considered significant if $P < 0.05$. Statistical analyses were performed using SPSS for Windows, Version 13.0 (SPSS Inc., Chicago, IL, U.S.).

RESULTS

Oxidative stress parameters in serum

Tables 1 to 3 depict the MDA level, SOD and GSH-Px activities in serum of three groups at 1, 2, 4, 8 and 12 weeks. At 1 week, MDA level in the three groups have no significant difference ($P > 0.05$). At 2 and 4 weeks, MDA

Table 1. The serum levels of MDA (nmol/ml) in three groups.

Group	1 week	2 weeks	4 weeks	8 weeks	12 weeks
Sham	19.82 ± 2.15	20.95 ± 2.32	17.79 ± 1.91	13.41 ± 1.79	13.37 ± 1.62
Injured	20.25 ± 2.11	21.16 ± 2.12	18.81 ± 1.98	14.61 ± 1.36	13.71 ± 1.67
LBP application	18.34 ± 2.02	16.04 ± 2.05*	14.13 ± 1.65*	13.02 ± 1.88	13.18 ± 1.79

*P<0.05 when compared with other two groups.

Table 2. The SOD (U/ml) activities in three groups.

Group	1 week	2 weeks	4 weeks	8 weeks	12 weeks
Sham	47.61 ± 12.35	50.12 ± 13.01	64.33 ± 12.98	67.25 ± 13.24	67.58 ± 13.16
Injured	44.23 ± 10.85	46.34 ± 11.27	59.92 ± 13.06	66.21 ± 12.13	67.75 ± 12.53
LBP application	78.34 ± 14.12*	87.28 ± 19.56*	92.75 ± 18.23*	73.04 ± 19.39	68.12 ± 14.36

*P<0.05 when compared with other two groups.

Table 3. The GSH-Px (U/ml) activities in three groups.

Group	1 week	2 weeks	4 weeks	8 weeks	12 weeks
Sham	125.36 ± 18.76	128.32 ± 14.96	140.33 ± 19.75	151.36 ± 19.41	151.47 ± 21.16
Injured	120.13 ± 18.49	124.59 ± 15.73	132.83 ± 18.71	150.53 ± 18.39	151.05 ± 19.31
LBP application	165.47 ± 19.28*	178.37 ± 22.54*	181.68 ± 21.43*	156.04 ± 20.03	152.12 ± 19.93

*P<0.05 when compared with other two groups.

levels in the LBP application group were significantly lower than those in the injured control and sham groups (P<0.01). At 1, 2, 4 week, SOD and GSH-Px activities in the LBP application group were significantly higher than those in the injured control and sham groups (P<0.01). At 8 and 12 weeks, MDA levels, SOD and GSH-Px activities in the three groups have no significant difference (P<0.05).

Functional parameters

To evaluate changes in erectile function, the mean peak ICP, which correlates with penile rigidity in male, was measured at 12 weeks. As shown in Figures 1 and 2, the injured control group had a significant reduction in peak ICP and peak ICP/MAP compared with the sham group, consistent with a state of erectile dysfunction. In the LBP application group, peak ICP and peak ICP/MAP were significantly higher than those in the injured control group (P<0.05), although lower than the sham group (P<0.05).

Histological parameters

The myelinated axons of CN were evaluated by toluidine blue staining of histological samples. As shown in Figure 3, the number of myelinated axons of CN in the injured

control group was significantly less than that in the sham group. The LBP application group had a significantly increase in the number of myelinated axons of CN compared with the injured control group (P<0.05), but still had less myelinated axons than the sham group (P<0.05). The histological results were in concordance with the functional results.

DISCUSSION

With the development of surgical techniques and scientific technology, nerve-sparing procedures and laparoscopic or robotic techniques have been used in radical prostatectomy. Nevertheless, considerable numbers of patients following radical prostatectomy has erectile dysfunction caused by CN injury, and many patients need use of medications such as phosphodiesterase-5 inhibitors for satisfactory erection (Woo et al., 2011). Recovery of erectile dysfunction requires CN regeneration as quickly as possible, otherwise the CN injury will leads to a progressive decrease in smooth muscle fibers and an increase in collagen fibers, eventually resulting in fibrosis of the corpus cavernosum. Therefore, how to accelerate CN regeneration has become a burning question that needs to be solved urgently.

Accumulating evidence indicates that the capability of

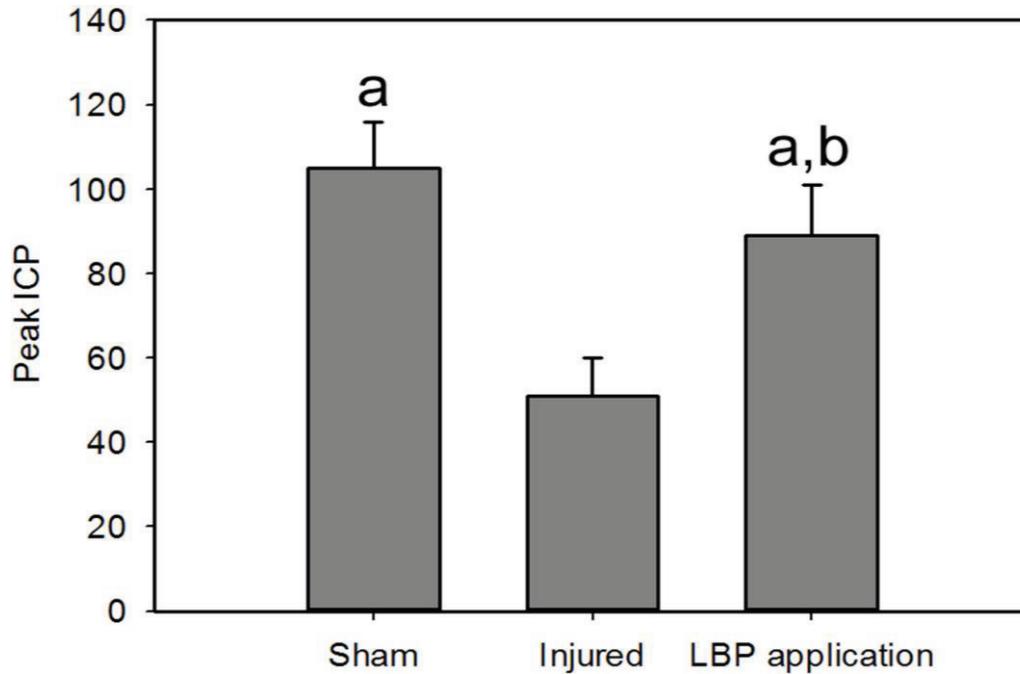


Figure 1. Peak ICP in three groups. ^aP<0.05 when compared with the injured group; ^bP<0.05 when compared with the sham group.

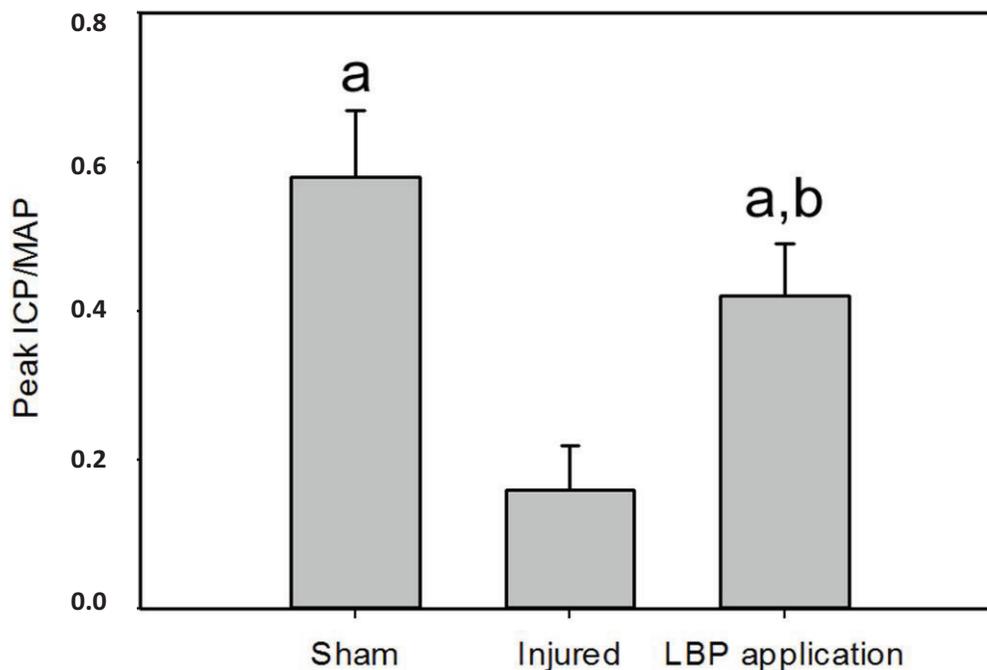


Figure 2. Peak ICP/MAP in three groups. ^aP<0.05 when compared with the injured group; ^bP<0.05 when compared with the sham group.

regeneration of axons is closely related to the microenvironment of injured area. Therefore, it is important to improve the local microenvironment for the CN regeneration (Webber and Zochodne, 2010). In this

microenvironment, there are two critical opposite factors to determine the conditions of CN regeneration. One is the neurotrophic cytokines for the positive effect, the other is trauma-induced oxidative stress for the negative effect

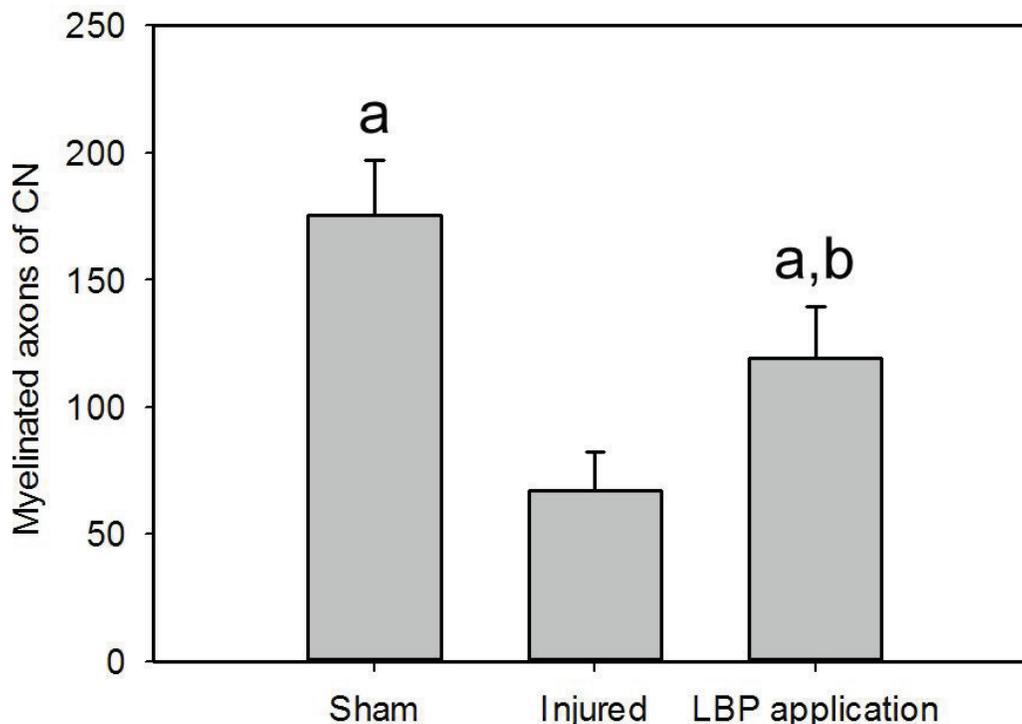


Figure 3. The number of myelinated axons of CN in three groups. ^aP<0.05 when compared with the injured group; ^bP<0.05 when compared with the sham group.

(Lagoda et al., 2007). There are several reports of erectile recovery after intracavernous injection with neurotrophic cytokines, such as neurturin, growth differentiation factor-5, platelet-rich plasma and insulin growth factor-1 (Bella et al., 2007; Fandel et al., 2008; Ding et al., 2009; Zhou et al., 2011). One study also reported that intracavernous injection with herpes simplex virus vector-mediated glial cell line-derived neurotrophic factor could improve nerve regeneration and erectile function in a rat model of bilateral CN injury (Kato et al., 2009). These studies aforementioned represented the beneficial role of neurotrophic cytokines in CN regeneration, as well as in the recovery of erectile function.

On the other hand, oxidative stress plays an inhibitory role in CN regeneration (Ozkara et al., 2006). Oxidative stress is associated with the generation of massive free radicals and reactive oxygen species (ROS), e.g. superoxide and hydrogen peroxide, and the decrease of antioxidant levels in target tissues and blood. The imbalance between oxidative reactions and antioxidant capacity might induce lipid peroxidation of polyunsaturated fatty acids in cell membranes, DNA damage, which results directly or indirectly in cellular damage. Antioxidants are substances that can reduce the severity of oxidative stress. Our study suggests that dietary antioxidants may prevent cellular damage because of their ability to detoxify abundant peroxides by scavenging ROS produced during oxidative stress.

In the last decades, considerable interest has developed in plants like *L. barbarum* which belongs to the Solanaceae family, commercially known as goji berry. It has been widely used as a popular functional food with generous biological activities and pharmacological functions, which can prevent and treat various diseases, such as diabetes, hyperlipidemia and cancer (Jing and Yin, 2010; Thomson, 2010; Miao et al., 2010; Mao et al., 2011). LBP extracted from *L. barbarum* generally consist of six monosaccharides (galactose, glucose, rhamnose, arabinose, mannose, and xylose). Previous studies have shown that LBP could resist cellular damage induced by oxidative stress and exhibit antioxidant activity *in vivo* (Amagase et al., 2009; Shan et al., 2011; Cheng and Kong, 2011). In our study, LBP as an antioxidant, can improve the antioxidant functions by both scavenging excessive free radicals and promoting antioxidant enzyme activities and can be used as therapeutic agent to CN injury.

In our study, oxidative stress was evaluated by serum levels of MDA, SOD activities and GSH-Px activities. MDA, a known biomarker of peroxidation and oxidative stress, has been widely used for assessing oxidative damage to lipids. Nevertheless, oxidative damage to proteins and DNA induces release of MDA. Our results showed that oxidative stress causes an increase in MDA in the three groups after surgical trauma. Antioxidant enzymes provide the primary defense against ROS

produced during oxidative stress. Superoxide dismutase reduces superoxide to form hydrogen peroxide; GSH-Px, one of antioxidant enzymes, reduces hydrogen peroxide to water. In addition, GSH-Px can reduce lipid peroxides directly. Therefore, it is reasonable to think that decreasing of MDA levels in LBP application group at 2 and 4 weeks may be partially due to a counteraction of the deleterious effects of lipid peroxidation, and increasing of SOD activities and GSH-Px activities in LBP application group at 1, 2 and 4 weeks might contribute to promote antioxidant enzyme activities for antagonizing the oxidative stress effects. The results strongly indicate that LBP plays an important role in trauma-induced oxidative stress.

The peak ICP, regulated by the status of CN, and peak ICP/MAP are two reliable indexes for evaluating erectile dysfunction (Woo et al., 2011; Ding et al., 2009; Bessede et al., 2010). The number of myelinated axons can reflect the effects of nerve regeneration histologically. Our results represent a measurable improvement on CN regeneration in the LBP application group based on the following results: (1) during CN electro-stimulation, the measurement of the peak ICP and peak ICP/MAP in this group showed good functional recovery; (2) histological evaluation of this group showed a significant increase in the number of myelinated axons, which indicated the recovery of CN directly.

In conclusion, this study shows that the application of LBP after CN injury improved the regeneration of CN and the recovery of erectile function. This positive effect could be attributed to the fact that LBP resists neuronal damage induced by oxidative stress both by scavenging free radicals and promoting antioxidant enzyme activities. Future work will include finding the detailed molecular mechanism of LBP in the process of nerve repair.

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ABBREVIATIONS

CN, Cavernous nerves; **LBP**, *Lycium barbarum* polysaccharides; **MDA**, malondialdehyde; **SOD**, superoxide dismutase; **GSH-Px**, glutathione peroxidase; **ICP**, intracavernous pressure; **MAP**, mean arterial pressure; **ROS**, reactive oxygen species.

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