Tropical Journal of Pharmaceutical Research December 2018; 17 (12): 2355-2363
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v17i12.6

Original Research Article

Myricetin promotes peripheral nerve regeneration in rat model of sciatic nerve injury via regulation of BDNF-Akt/GSK-3β/mTOR signalling pathway

Dechou Zhang[1], Jianhua Ge[2]*, Lisha Ye[1], Hongmei Tang[1], Xue Bai[1]
[1]Department of Cardiology and Encephalopathy, Affiliated Traditional Chinese Medicine Hospital of Southwest Medical University, [2]Department of Bone and Joint, Affiliated Hospital of Southwest Medical University, Luzhou, Sichuan, 646000, PR China

For correspondence: **Email: JessenParkertj@yahoo.com; **Tel:** 0086–8302292219*

Sent for review: 13 June 2018 *Revised accepted: 21 November 2018*

Abstract

Purpose: *To investigate the effects of myricetin on peripheral nerve regeneration in sciatic nerve crush injury model.*
Methods: *Separate groups of rats were administered myricetin at 25, 50 or 100 mg/kg body weight/day for 2 weeks. Functional recovery following sciatic nerve injury was assessed by foot position and walking track analyses, measurement of mechanical hyperalgesia, and withdrawal reflex latency (WRL).*
Results: *Myricetin treatment resulted in significantly enhanced recovery of sensorimotor functions as evidenced by increased scores in functional analysis tests. Myricetin treatment remarkably elevated brain derived neurotrophic factor (BDNF) expression, and also enhanced activation of Akt and mTORc1, reflecting up-regulation of PI3K/Akt/mTORC1 signalling involved in nerve regeneration.*
Conclusion: *Myricetin enhances functional recovery and nerve regeneration in rats. These findings suggest that myricetin is a potent neuroprotective agent with potential for the management of peripheral nerve injury.*

Keywords: *Glycogen synthase kinase 3β, Mammalian target of rapamycin (mTOR), Myricetin, Nerve regeneration*

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

In clinical practice, peripheral nerve injuries (PNIs) are encountered frequently due to tumours, accidental trauma, acute compression, surgeries or iatrogenic injuries. Traumatic injuries are on the increase, with estimated 500,000 new patients every year [1]. The peripheral nerve damage results in total or partial function impairment affecting the motor, sensory and autonomic functions. Peripheral nerve injury (PNI) is characterized by disruption of axons and myelin sheaths [2]. Nevertheless, Schwann cell tubes of the basal lamina remain intact and aid in regeneration of axons and re-innervation of tissues [3,4]. However, the time taken for re-innervation of the target tissues is critical for improvement in sensory and motor functions, which makes accelerated regeneration vital for adequate functional recovery [5]. Sciatic nerve

crush injury is an adequate experimental model for studying the molecular events involved in peripheral nerve regeneration. The model is extensively employed in assessing the effectiveness of novel drugs in enhancing the speed of regeneration and effectively improving re-innervation [1].

The pivotal part of neurotrophins in neuroprotection and neuro-regeneration is well documented. The neurotrophic factors (NTFs) regulate neuronal survival, viability and differentiation [6]. Following peripheral nerve injury, Schwann cells (SCs) aid in the removal of injured end of the axons. Several neurotrophic factors such as nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), glial cell derived neurotrophic factor (GDNF), and neurotrophic factors-3, -4/5 and -6 are secreted by SCs [7, 8]. Studies have demonstrated that when neurotrophic factors are transported to the site of PNI, *in vivo* nerve regeneration is improved [7,9].

The mechanistic target-of-rapamycin (mTOR), a serine-threonine protein kinase, is critically involved in vital signaling pathways. It is the core constituent of mTOR complex 1 (mTORC1) and 2 (mTORC2). Studies have shown that mTORC1 is crucial for regulating various anabolic reactions [10], and is tightly associated with the major cell signaling pathway, viz, PI3K-Akt pathway. PI3K-Akt signaling, a major downstream target of growth factors, controls a wide range of functions including cell survival, proliferation, growth, and metabolism. Activation of mTORC1 regulates phosphatase and tensin homolog (*PTEN), the chief inhibitor of* PI3K/Akt [11]. In addition, AKT phosphorylates and inhibits glycogen synthase kinase 3β (GSK-3β, another key downstream protein) which is critical for axon growth and branching [12]. Studies have reported that the Akt-mTORC1 pathway plays a pivotal role in PNS and CNS myelination [13, 14]. Compounds that can regulate the PI3K-Akt-mTORC1 cascade would be of immense clinical value.

Myricetin (3, 3', 4', 4, 5, 5', 7 - hexahydroxyflavone), a plant-derived flavonoid is present in various vegetable, fruits, nuts and red wine [15]. Myricetin has been reported to possess anti-oxidant and antibacterial [16]; cardio-protective [17], and anti-proliferative properties [18]. The lipid-lowering activities of myricetin [19], and its anti-diabetic properties [20] have been reported. However, not much work has been done on the effects of myricetin on nerve regeneration. In this study, the effect of myricetin on enhancement of nerve regeneration following PNI in sciatic nerve crush injury model was investigated.

EXPERIMENTAL

Antibodies and chemicals

Myricetin was procured from Sigma-Aldrich (St. Louis, MO, USA). Antibodies against BDNF, TrkB, Akt, mTORc1, PTEN, GSK-3β, phospho-Akt, phospho- GSK-3β, and phospho-mTORC1 were also procured from Sigma-Aldrich. β-Actin and proliferating cell nuclear antigen (PCNA) used for expression analysis was purchased from Santa Cruz Biotechnology (CA, USA). All other chemicals used were of analytical grade and were obtained from Sigma-Aldrich unless otherwise stated.

Experimental animals

Adult Sprague-Dawley rats weighing 200 - 220 g were procured from research laboratory animal facility of our University. The animals were housed in sterile polypropylene cages (2 per cage) under standard animal house settings (23 ± 2 ℃; 12-h dark/12-h light cycle, and 50 - 55 % relative humidity), with unrestricted access to feed and clean drinking water. The animal experimental protocols were approved by the ethical committee of Southwest Medical University (approval number: TK345511), and were carried out in compliance with international animal welfare guidelines [21].

Surgical procedure for sciatic nerve injury

The rats were anesthetized by intraperitoneal (*i.p.*) injection of 0.7 mg/kg acepromazine, 10 mg/kg xylazine, and 95 mg/kg ketamine. The surgery sites were shaved neatly and wiped with alcohol (70 %). Using the gluteal muscle splitting method, the sciatic nerve was uncovered by incision made along the mid-thigh level with the help of a surgical microscope. At 10 mm from the sciatic notch, the sciatic nerve was crumpled for a total of 30 sec (10 sec at a time) with a pause of 10 s [22]. The sciatic nerve was observed to make sure that the epineurial sheath was undamaged but translucent due to injury (axotomy). It was reimbursed back below the muscle [23], and the incision was sutured.

The rats exposed to sciatic nerve crush injury were randomly divided into 5 groups (groups II to V) with 12 rats/group. Treatment groups (groups III - V) received myricetin (25, 50 or 100 mg/kg body weight) via oral gavage every day for 14 days, starting 6 h after sciatic nerve crush damage. Group I rats (control) were not

subjected to nerve injury. Groups I and II rats were treated with equivalent volumes of saline in place of myricetin.

Evaluation of functional recovery

Foot positioning

Following sciatic nerve injury, foot positioning was monitored. The animals were positioned on a plate at room temperature, and foot positioning was observed every 3rd day following 24 h post-injury for 21 days. The foot positioning of the rats was scored based on altered locomotor scale ranging from 1 to 3 [24].

Measurement of withdrawal reflex latency

Withdrawal reflex latency (WRL) refers to the time taken for the withdrawal of the affected hind paw from a hotplate. For the measurement of WRL, animals with the injured hind paws were placed on a hotplate (at 56 ℃) after wrapping the rats with surgical cloth above the midriff. This test was conducted thrice with a break of 120 sec between repeated tests to avoid sensitization [25]. The latency time was determined with a stopwatch, and was measured from the time the rats were placed on the plate to the time of withdrawal of the hind paw [26].

Mechanical hyperalgesia

To measure hyperalgesia following sciatic nerve crush damage, each rat was placed gently on a non-greasy surface, and onto the dorsal surface of the affected hind limbs, a piston was applied using computerized gauge. The display of pain by either making a noise or paw withdrawal was recorded. When animals exhibited pain, the pressure due to the device was released proximately and pain threshold was recorded by means of an Analgesic Meter (Model 21025, UGO Basile, Italy) [27]. The mean of three successive values punctuated by an interval of 10 - 20 min was determined. The pressure needed to induce withdrawal of paw in the animals is called the paw pressure threshold. Pain threshold levels were measured every 5 days and expressed in grams according to the method of Renno *et al* [24].

Determination of motor function recovery

For determination of recovery of locomotor activity, sciatic functional index (SFI) [28] and toe spread index (TSI) [29] assessments were conducted. The indices were constructed on measurements of walking footpaths which reflect functional sciatic nerve recovery.

For assessing locomotor activity, rats were trained to move on a white sheet of paper that covered the bottom of an 8.5-cm wide and 100-cm long track in a dark box [30]. Later, the hind feet of the animals were dipped in red dye to track the walk. To determine the following measurements, footprints of rats were used: Print length (PL) - the distance from the heel to the third toe; toe spread (TS) - the distance between the first and fifth toes, and intermediary toe spread (ITS) - the distance between the second and the fourth toes. The measurements were recorded for the affected limb (E) and normal (N) limb. For TSI assessment, TS was measured. Multiple prints from each foot were acquired, but only three prints were used to determine the mean extents in the E and N sides. The index of nerve dysfunction, SFI, ranges from 0 to 100, with 0 equivalent to regular function, and 100 equivalent to wide-range dysfunction. It was calculated using Eq 1.

SFI = - 38:3(EPL - NPL)/NPL + 109.5 (ETS-NTS)/NTS + 13.3(EIT- NIT)/NIT- 8:8 …. (1)

TSI was computed as in Eq 2 [31].

TSI = (ETS - NTS)/NTS …….. (2)

Western blot analysis

The sciatic nerve tissues (6 per group) were normalized in cell lysis buffer and centrifuged for 30 min at 4 ℃ [32]. Total protein content of the supernatant was measured using Bradford assay (Bio-Rad). Equivalent quantities of the protein samples (50 µg) were subjected to SDS-polyacrylamide gel electrophoresis SDS-PAGE). The protein bands were blotted and transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore). The membranes were then blocked with non-fat milk (5 %) in TBST (20 mM Tris, 0.05 % Tween-20, and 150 mM NaCl) for 2 h at normal temperature. Thereafter, the PVDF membranes were washed with PBS and incubated at 4 ℃ with primary antibodies overnight followed by incubation with horseradish peroxidase-conjugated secondary antibody at room temperature for 60 min. The blots were washed further and the immune-reactive bands were quantified using enhanced chemiluminescence method (GE Healthcare, Piscataway, NJ).

RT-PCR analysis

In order to determine the gene expressions of TrkB and BDNF, RT-PCR analysis was performed. Total RNA was extracted from nerve

tissues by means of TRIzol (Invitrogen, Carlsbad, CA, USA) as per manufacturer's guidelines. The RNA samples were then exposed to DNaseI digestion (Qiagen, Inc. Valencia, CA) to exclude any DNA residues, and the RNA density was assessed (Nanodrop spectrophotometer - ND-1000, Bio-Rad, USA). The initial strand of cDNA was created by reverse transcription using Fermentas Company (USA) Revert Aid First Strand cDNA Synthesis Kit. The PCR procedures were carried out as directed in the kit protocol (Fermentas Company, USA) using the following primer sequences for BDNF and TrkB:

BDNF forward: 5'-CGAAGAGCTGCTGGATGAG-3, reverse: 5'-ATGGGATTACACTTGGTCTCG-3; TrkB forward: 5'-CCTCCACGGATGTTGCTGA-3', reverse: 5'-GGCTGTTGGTGATACCGAAGTA-3' GADPH forward: 5'-CCGTATCGGACGCCTGGTTA-3', reverse: 5'-GGCTGTTGGTGATACCGAAGTA-3.

GAPDH expression was used as the internal control. The PCR mixture obtained was run on agarose gel (1 %) and the products were visualised with ethidium bromide (0.05 %). Band intensities were scanned and analysed with Bio-Gel imagery apparatus (Bio-Rad, USA).

Statistical analysis

The results of the study are presented as mean ± standard deviation (SD) obtained from six independent trials. Statistical differences between different groups were analysed using SPSS package (version 22.0). The results were subjected to one-way analysis of variance (ANOVA) with subsequent post-hoc analysis using Duncan's multiple range test. Values of $p < 0.05$ were assumed to be statistically significant.

RESULTS

Myricetin treatment enhanced functional recovery after nerve crush injury

The effects of myricetin action on functional recovery following sciatic nerve crush injury were assessed. The foot location analysis revealed a progressive retrieval in the animals treated with myricetin (25, 50 or 100 mg/kg). Myricetin-treated group exhibited marked recovery on day 21 post-crush injury, relative to the saline-treated injured rats. Interestingly, rats treated with higher dose of myricetin (100 mg/kg) presented negligible foot location movement or any irregular foot placing from day 14 post-surgery through day 21 (Figure 1).

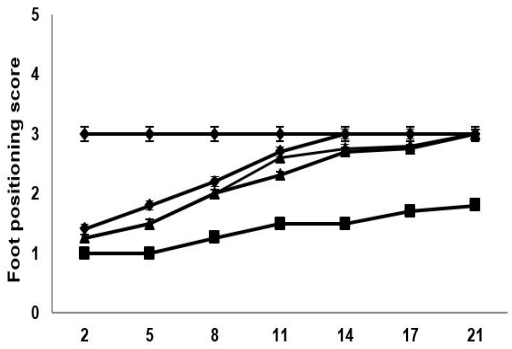

Figure 1: Myricetin enhanced motor function recovery following sciatic nerve crush injury. ♦ = control; ■ = sciatic nerve crush (SNC) injury; ▲ = SNC + 25 mg myricetin; ▲ = SNC + 50 mg myricetin; ♦ = SNC + 100 mg myricetin. Values are represented as mean ± SD (n = 6)

Myricetin improved withdrawal reflex latency

Withdrawal reflex latency values reflect the nociceptive and motor functions of peripheral nerves. The latency values were higher in the saline-treated injured group than in the normal control group. Myricetin administration significantly ($p < 0.05$) reduced the latency values, when compared to group II animals. Furthermore, the latency values of rats treated with 100 mg myricetin on days 8, 12 post-injury were similar to latency values of normal control rats (Figure 2). These observations suggest that myricetin treatment enhances recovery of motor and nociceptive functions of injured sciatic nerve.

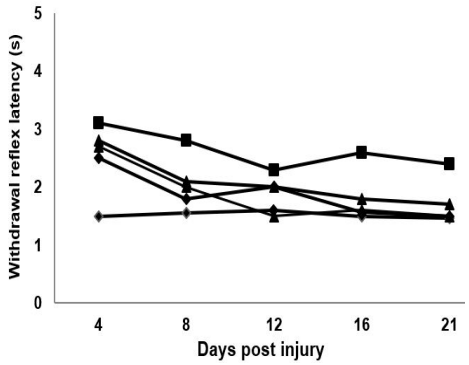

Figure 2: Myricetin enhanced withdrawal reflex latency following sciatic nerve crush injury. ♦ = control; ■ = sciatic nerve crush (SNC) injury; ▲ = SNC + 25 mg myricetin; ▲ = SNC + 50 mg myricetin; ♦ = SNC + 100 mg myricetin. Values are represented as mean ± SD (n = 6)

Effect of myricetin on withdrawal reflex thresholds

The withdrawal reflex thresholds (WRT) of affected hind limb in response to external pressure stimulus was measured and is termed

powered hyperalgesia. It was measured every week following injury, for 3 weeks. On the 7th day following SNC injury, the rats displayed higher WRT values, when compared to normal control rats. On the 2nd and 3rd weeks, there was a slight decrease in mechanical hyperalgesia in the saline-treated injured rats, when compared to the 1st week. Nevertheless, the myricetin-treated rats presented a marked decrease in paw WRT to powered stimulus, relative to saline-treated crush group from 14th to 21st day post-injury (Figure 3). There was no considerable difference in WRT values between rats treated with 100 mg myricetin and rats in the normal control group.

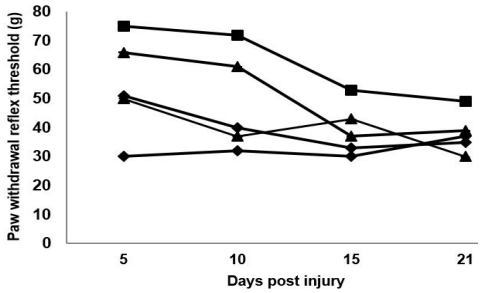

Figure 3: Effects of myricetin on nociceptive mechanical thresholds. ♦ = control; ■ = sciatic nerve crush (SNC) injury; ▲ = SNC + 25 mg myricetin; ▲ = SNC + 50 mg myricetin; ♦ = SNC + 100 mg myricetin. Values are represented as mean ± SD (n = 6)

Myricetin enhanced motor function recovery

In the normal control-saline treated animals, SFI and TSI values were normal, with the SFI value around -2, and TSI value zero. However, the TSI and SFI values in injured-saline treated rats were significantly reduced (Table 1 and Table 2), indicating functional impairment following nerve crush injury. Interestingly, treatment with myricetin produced recovery of sensorimotor function as evidenced by significant ($p < 0.05$) increases in SFI and TSI values. On day 21, the SFI scores increased from -40.21 ± 1.91 to -13.16 ± 0.81; while TSI increased to -0.09 ± 0.001 from -0.29 ± 0.011 on treatment with myricetin at 100 mg. These observations demonstrate that myricetin enhanced recovery of sensorimotor functions following SNC injury.

Myricetin raised BDNF-TrkB expression

The mRNA levels of BDNF and TrkB in the crushed nerves were determined using RT-PCR. Following SNC injury, the BDNF and TrkB mRNA levels were significantly reduced, when compared to normal control rats ($p < 0.05$). However, the BDNF expression and TrkB mRNA increased significantly in myricetin-treated SNC injury rats (Figures 4 A and B), relative to the saline-treated SNC injure rats. The raised levels of BDNF expression and TrkB mRNA reflect increased nerve regeneration following myricetin treatment, since BDNF is pivotal in regulating and controlling neuronal cell growth, survival and proliferation.

Myricetin up-regulated Akt/mTORC1 signalling

The effects of myricetin on Akt/mTORC1 signalling following SNC injury was assessed. Following SNC injury, noticeably raised levels of phosphorylated forms of Akt, mTORc1 and GSK-3β was observed (Figure 5 A - C).

Table 1: Sciatic functional index

Group	Days post-injury			
	1	7	14	21
Control	-10.20 ± 1.2 [b]	-15.11 ± 1.27 [b]	-12.01 ± 0.82 [b]	-12.07 ± 1.39 [b]
Sciatic nerve crush (SNC) injury	-48.71 ± 1.7 [ad]	-45.36± 1.90 [ae]	-50.05 ± 3.85 [ad]	-40.21 ± 1.91 [ad]
SNC + 25 mg myricetin	-32.38 ± 2.03 [ac]	-39.22 ± 2.73 [ad]	-35.19 ± 3.21 [ac]	-19.77 ± 0.37 [ac]
SNC + 50 mg myricetin	-38.00 ± 1.32 [ac]	-32.10 ± 1.97 [ac]	-30.87 ± 2.00 [ac]	-15.82 ± 1.26 [ac]
SNC + 100 mg myricetin	-33.26 ± 2.76 [ac]	-29.15 ± 1.02 [ac]	-31.37 ± 0.97 [ac]	-13.16 ± 0.81 [ac]

Values are expressed as mean ± SD; n = 6; [a]$p < 0.05$, compared to control; [b-e]$p < 0$ between mean values for experimental groups on a particular day

Table 2: Toe spread index

Group	Post-injury period (days)			
	1	*7*	*14*	*21*
Control	0.01 ± 0.001 [b]	0.02 ± 0.001 [b]	0.00 ± 0.00 [b]	0.07 ± 0.002 [b]
Sciatic Nerve Crush (SNC) injury	-0.37 ± 0.010 [ad]	-0.45 ± 0.009 [ad]	-0.70 ± 0.042 [ad]	-0.29 ± 0.011 [ae]
SNC + 25 mg myricetin	-0.22 ± 0.030 [ac]	-0.29 ± 0.010 [ac]	-0.39 ± 0.021 [ac]	-0.20 ± 0.010 [ad]
SNC + 50 mg myricetin	-0.29 ± 0.002 [ac]	-0.31 ± 0.007 [ac]	-0.30 ± 0.011 [ac]	-0.11 ± 0.021 [ac]
SNC + 100 mg myricetin	-0.26 ± 0.006 [ac]	-0.29 ± 0.021 [ac]	-0.37 ± 0.009 [ac]	-0.09 ± 0.001 [ac]

Values are expressed as mean ± SD (n = 6); [a]$p < 0.05$, compared to control; [b-e]$p < 0.05$, between mean values for experimental groups on a particular day

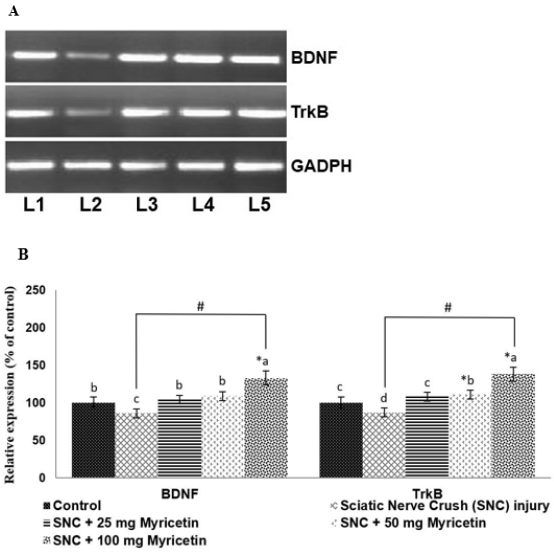

Figure 4: Myricetin regulated the expressions of BDNF and TrkB. A: Representative RT-PCR Gel; B: Relative expression of proteins. Data are represented as mean ± SD (n = 6). *p < 0.05, compared with control; #p < 0.05, SNC compared with SNC + 100 mg myricetin (a, b, c & d represent mean values from different experimental groups that differ significantly)

However, myricetin treatment significantly (p < 0.05) enhanced phosphorylated forms of Akt, mTORc1 and GSK-3β. Markedly reduced PTEN expressions were seen in myricetin treated rats (p < 0.05), indicating activation of the pathway. The levels of total Akt, mTORc1 and GSK-3β were not altered, although slightly raised expressions were noticed on myricetin treatment. These results suggest possible activation of the PI3K/Akt/mTORC1 signalling by myricetin.

DISCUSSION

Peripheral nerve injuries following accidental trauma and sport injuries are frequently seen in clinical practice worldwide. Post-traumatic peripheral nerve regeneration and functional recovery pose a huge challenge since inadequate restoration of function and innervation leads to subsequent loss of function of the target organ [33].

Thus, accelerated repair of the damaged nerves, enhanced regeneration of nerves and sensorimotor functional recovery are crucial in restorative medicine. Sciatic nerve crush injury prototype is a widely employed animal model in studies of regeneration of nerves [34]. In this study, the effects of myricetin on nerve regeneration and functional recovery following sciatic nerve crush injury were investigated.

It was observed that myricetin treatment effectively improved the foot positioning of the rats.

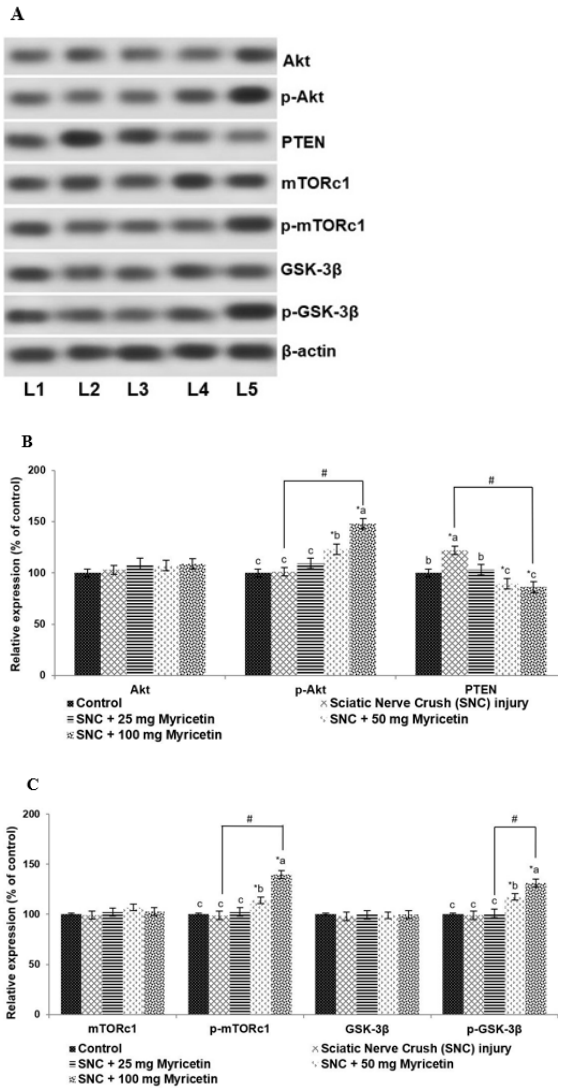

Figure 5: Myricetin up-regulated Akt activation following SNC injury. A: Representative immunoblot; B: Relative expression of proteins following SNC injury. Data are presented as mean ± SD (n = 6). *p < 0.05, compared with control; #p < 0.05 (SNC compared with SNC + 100 mg myricetin; a-c represent mean values from different experimental groups that differ significantly)

The WRL and WRT values reflect the beneficial effects of myricetin on enhancement of functional recovery of the sciatic nerve, while SFI and TSI values obtained following rat walking track analysis reflect the motor function of the injured hind limb. The SFI values are regarded as a standard index for evaluating motor function of nerves following nerve crush injury and repair [35]. In this study, all the experimental groups exhibited gradual recovery of sciatic nerve function. Interestingly, the myricetin-treated rats showed significantly higher SFI and TSI values than the saline treated-injured group. The

recovery of sensorimotor functions was enhanced and myricetin-treated injured rats presented SFI very close to that of normal control rats on the 3rd week post-injury. The SFI and TSI values reflect gain in function following SNC injury.

It is well documented that the neurotrophic factors are crucial in neuronal cell proliferation and growth following nerve injuries [36]. The BDNF is a major neurotrophic factor that is involved in axonal regeneration and myelinogenesis of injured nerve cells. Studies have demonstrated that BDNF increased axonal regeneration and myelination in nerve fibres following sciatic nerve crush [37]. In this study, myricetin treatment clearly enhanced BDNF and TrkB mRNA expressions. The raised levels of BDNF were in line with increased axonal regeneration and myelination as observed. The increase in BDNF levels could have, in part enhanced nerve regeneration observed following myricetin treatment.

In injured nerve cells, multiple factors are associated with the process of repair and regeneration. The PI3K/AKT/mTORC1 pathway is the major pathway involved in nerve cell growth, proliferation and survival [38]. Studies have shown that activation of the mTORC1 pathway and deletion of inhibitor PTEN leads to enhanced axonal regeneration, and AKT activation triggers regeneration of axon fibres at the site of crush injury [38]. A marked up-regulation in the levels of phosphorylated AKT and mTORC1 along with reduced PTEN expressions was observed in this study. Furthermore, elevated expression of phosphorylated forms of GSK3β were observed on myricetin treatment. The GSK3 family comprises GSK3α and GSK3β. It is known that AKT phosphorylates and inactivates the kinase activity of GSK3α at Ser21 or GSK3β at Ser9 [39]. Interestingly, GSK3β inactivation has been shown to mediate AKT signalling-induced axon regeneration [40]. Thus, the results obtained in this study suggest activation of Akt leading to raised levels of p-mTORC1 and p- GSK3β, and supressed PTEN levels. The up-regulation of the pathway could have subsequently led to raised nerve regeneration.

CONCLUSION

The results obtained in this study reveal the effectiveness of myricetin in enhancing functional recovery and nerve regeneration following peripheral nerve injury. Myricetin activates the BDNF and PI3K/AKT/mTORC1/GSK3β signalling pathways which are crucial in nerve cell growth. The findings reveal the effectiveness of myricetin in enhancing functional recovery and nerve regeneration following peripheral nerve injury via activation of the BDNF and PI3K/AKT/mTORC1/GSK3β signalling pathways involved in nerve cell growth.

DECLARATIONS

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the author(s) - Dechou Zhang#, Jianhua Ge#, Lisha Ye, Hongmei Tang, Xue Bai - and all liabilities pertaining to claims relating to the content of this article will be borne by the authors". Dechou Zhang and Jianhua Ge have equally contributed in designing the study, collected and analysed the data, and prepared the manuscript. Lisha Ye and Hongmei Tang contributed in the experimental works and Xue Bai had contributed in statistical study and all authors had contributed in all aspects of this research.

REFERENCES

1. *Rodríguez FJ, Valero-Cabré A, Navarro X. Regeneration and functional recovery following peripheral nerve injury. Drug Discovery Today: Disease Models 2004; 1: 177-185.*
2. *Sheng QS, Wang ZJ, Zhang J, Zhang YG. Salidroside promotes peripheral nerve regeneration following crush injury to the sciatic nerve in rats. Neuroreport 2013; 24: 217-223.*
3. *Mazzer PY, Barbieri CH, Mazzer N, Fazan VP. Morphologic and morphometric evaluation of experimental acute crush injuries of the sciatic nerve of rats. J Neurosci Methods 2008; 173: 249-258.*
4. *Wood MD, Kemp SW, Weber C, Borschel GH, Gordon T. Outcome measures of peripheral nerve regeneration. Ann Anat 2011; 193: 321-333.*
5. *Yuan Y, Shen H, Yao J, Hu N, Ding F, Gu X. The protective effects of Achyranthes bidentata polypeptides in an experimental model of mouse sciatic nerve crush injury. Brain Res Bull 2010; 81: 25-32.*
6. *Parkash V, Lindholm P, Peränen J, Kalkkinen N, Oksanen E, Saarma M, Leppänen VM, Goldman A. The structure of the conserved neurotrophic factors MANF and CDNF explains why they are bifunctional. Protein Eng Des Sel 2009; 22: 233-241.*
7. *Frostick SP, Yin Q, Kemp GJ. Schwann cells, neurotrophic factors, and peripheral nerve regeneration. Microsurgery 1998; 18: 397-405.*

8. Huo DS, Zhang M, Cai ZP, Dong CX, Wang H, Yang ZJ. The role of nerve growth factor in ginsenoside rg1-induced Regeneration of injured rat sciatic nerve. J Toxicol Environ Health A 2015; 78: 1328-1337.
9. Terenghi G. Peripheral nerve regeneration and neurotrophic factors. J Anat 1999; 194: 1-14.
10. Ben-Sahra I, Howell JJ, Asara JM, Manning BD. Stimulation of de novo pyrimidine synthesis by growth signaling through mTOR and S6K1. Science 2013; 339: 1323-1328.
11. Manning BD, Cantley LC. AKT/PKB Signaling: Navigating Downstream. Cell 2007; 129: 1261-1274.
12. Kim YT, Hur EM, Snider WD, Zhou FQ. Role of GSK3 signaling in neuronal morphogenesis. Front Mol Neurosci 2011; 4: 48.
13. Taveggia C. Schwann cells-axon interaction in myelination. Curr Opin Neurobiol 2016; 39: 24-29.
14. Wood TL, Bercury KK, Cifelli SE, Mursch LE, Min J, Dai J, Macklin WB. mTOR: a link from the extracellular milieu to transcriptional regulation of oligodendrocyte development. ASN Neuro 2013; 5: e00108.
15. Ross JA, Kasum CM. Dietary flavonoids: Bioavailability, metabolic effects, and safety. Annu Rev Nutr 2002; 22: 19-34.
16. Zhang D, Xie L, Jia G, Cai S, Ji B, Liu Y, Wu W, Zhou F, Wang A, Chu L, et al. Comparative study on antioxidant capacity of flavonoids and their inhibitory effects on oleic acid-induced hepatic steatosis in vitro. Eur J Med Chem 2011; 46: 4548-4558.
17. Lian TW, Wang L, Lo YH, Huang IJ, Wu MJ. Fisetin, morin and myricetin attenuate CD36 expression and oxLDL uptake in U937-derived macrophages. Biochim Biophys Acta. 2008; 1781: 601-609.
18. Zhang XH, Chen SY, Tang L, Shen YZ, Luo L, Xu CW, Liu Q, Li D. Myricetin induces apoptosis in HepG2 cells through Akt/p70S6K/bad signaling and mitochondrial apoptotic pathway. Anticancer Agents Med Chem 2013; 13: 1575-1581.
19. Bhatia G, Khanna AK, Sonkar R, Mishra SK, Srivastava S, Lakshmi V. Lipid lowering and antioxidant activity of flavones in triton treated hyperlipidemic rats. Med Chem Res 2011; 20: 1622-1626.
20. Ong KC, Khoo HE. Effects of myricetin on glycemia and glycogen metabolism in diabetic rats. Life Sci 2000; 67: 1695-1705.
21. Garber JC (Chair). Committee for the Update of the Guide for the Care and Use of Laboratory Animals. Guide for the Care and Use of Laboratory Animals, eighth ed. Washington DC, USA: National Academy of Sciences; 2011.
22. Chen L, Qin J, Cheng C, Niu S, Liu Y, Shi S, Liu H, Shen A. Spatiotemporal expression of SSeCKS in injured rat sciatic nerve. Anat Rec (Hoboken). 2008; 291: 527-537.
23. Varejão AS, Cabrita AM, Meek MF, Bulas-Cruz J, Melo-Pinto P, Raimondo S, Geuna S, Giacobini-Robecchi MG. Functional and morphological assessment of a standardized rat sciatic nerve crush injury with a non-serrated clamp. J Neurotrauma 2004; 21: 1652-1670.
24. Renno WM, Saleh F, Klepacek I, Al-Khaledi G, Ismael H, Asfar S. Green tea pain modulating effect in sciatic nerve chronic constriction injury rat model. Nutr Neurosci 2006; 9: 41-47.
25. Luís AL, Amado S, Geuna S, Rodrigues JM, Simões MJ, Santos JD, Fregnan F, Raimondo S, Veloso AP, Ferreira AJ, et al. Long-term functional and morphological assessment of a standardized rat sciatic nerve crush injury with a non-serrated clamp. J Neurosci Methods 2007; 163: 92-104.
26. Masters DB, Berde CB, Dutta SK, Griggs CT, Hu D, Kupsky W, Langer R. Prolonged regional nerve blockade by controlled release of local anesthetic from a biodegradable polymer matrix. Anesthesiol 1993; 79: 340-346.
27. Courteix C, Bardin M, Chantelauze C, Lavarenne J, Eschalier A. Study of the sensitivity of the diabetes-induced pain model in rats to a range of analgesics. Pain 1994; 57: 153-160.
28. Teodosijevic M, Miljkovic Z, Majstorovic Z. Use of the maximal physiologic load test in the evaluation of functional values of newly made complete dentures and dentures rebased by the Rehm method. Vojnosanit Pregl 1998; 55: 161-170.
29. Smit X, de Kool BS, Blok JH, Visser GH, Hovius SE, van Neck JW. Recovery of neurophysiological features with time after rat sciatic nerve repair: a magneto-neurographic study. J Peripher Nerv Syst 2006; 11: 126-134.
30. Sacharuk VZ, Lovatel GA, Ilha J, Marcuzzo S, Pinho AS, Xavier LL, Zaro MA, Achaval M. Thermographic evaluation of hind paw skin temperature and functional recovery of locomotion after sciatic nerve crush in rats. Clinics 2011; 66: 1259-1266.
31. Kinnman E, Aldskogius H, Johansson O, Wiesenfeld-Hallin Z. Collateral reinnervation and expansive regenerative reinnervation by sensory axons into ‘‘foreign’’ denervated skin: an immunohistochemical study in the rat. Exp Brain Res 1992; 91: 61-72.
32. Yuan D, Shen J, Yan Y, Wu X, Li A, Guo A, Wu Y, Duan C, Shen J, Tang C, et al. Upregulated expression of SSTR1 is involved in neuronal apoptosis and is coupled to the reduction of bcl-2 following intracerebral hemorrhage in adult rats. Cell Mol Neurobiol 2014; 34: 951-961.
33. Chen XD, Gu YD. Study progresses in peripheral nerve injury and motor neuoral cell death. Chin J Microsurg 2000; 23: 238-240.
34. Jiang M, Zhuge X, Yang Y, Gu X, Ding F. The promotion of peripheral nerve regeneration by chitooligosaccharides in the rat nerve crush injury model. Neurosci Lett 2009; 454: 239-243.
35. Schiaveto de Souza A, da Silva CA, Del Bel EA. Methodological evaluation to analyze functional recovery after sciatic nerve injury. J Neurotrauma 2004; 21: 627-635.

36. Hausner T, Nógrádi A. The use of shock waves in peripheral nerve regeneration: New perspectives? Int Rev Neurobiol 2013; 109: 85-98.
37. Alrashdan MS, Sung MA, Kwon YK, Chung HJ, Kim SJ, Lee JH. Effects of combining electrical stimulation with BDNF gene transfer on the regeneration of crushed rat sciatic nerve. Acta Neurochirurgica 2011; 153: 2021-2029.
38. Park KK, Liu K, Hu Y, Smith PD, Wang C, Cai B, Xu B, Connolly L, Kramvis I, Sahin M, et al. Promoting axon regeneration in the adult CNS by modulation of the PTEN/mTOR pathway. Sci 2008; 322: 963-966.
39. Cross DA, Alessi DR, Cohen P, Andjelkovich M, Hemmings BA. Inhibition of glycogen synthase kinase-3 by insulin mediated by protein kinase B. Nature 1995; 378: 785-789.
40. Guo X, Snider WD, Chen B. GSK3β regulates AKT-induced central nervous system axon regeneration via an eIF2Bε-dependent, mTORC1-independent pathway. Elife 2016; 5: e11903.