British Journal of Medicine & Medical Research
8(9): 781-789, 2015, Article no.BJMMR.2015.506
ISSN: 2231-0614

SCIENCEDOMAIN *international*
www.sciencedomain.org

Potential Neuroprotective Role of Verapamil in Experimentally- Induced Chronic Sciatic Nerve Constriction in Mice

Inas El Sayed Darwish[1*] and Iman Samy Dessouky[2]

[1]Department of Clinical Pharmacology, Faculty of Medicine, Al Moassat Medical Campus, Alexandria University, Egypt.
[2]Department of Medical Biochemistry, Faculty of Medicine, Al Moassat Medical Campus, Alexandria University, Egypt.

Authors' contributions

This work was carried out in collaboration between both authors. Author IESD designed the study, wrote protocol, managed the experimental process and wrote the first draft of manuscript. Author ISD managed the literature searches, analysis of biochemical measures. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJMMR/2015/17908
Editor(s):
(1) Sinan INCE, Department of Pharmacology and Toxicology, University of Afyon Kocatepe, Turkey.
Reviewers:
(1) Anonymous, Universidad Veracruzana, Mexico.
(2) Daniela Hanganu, Pharmacognosy Department, Iuliu Hatieganu University, Romania.
(3) Anonymous, Usmanu Danfodiyo University, Nigeria.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=1121&id=12&aid=9441

Original Research Article

Received 30th March 2015
Accepted 13th May 2015
Published 27th May 2015

ABSTRACT

Objectives: The aim of the current study was to evaluate of three dose levels of verapamil; as a calcium channel blocker that could confer an anti-inflammatory efficacy, in an experimental model of neuropathy in mice that had been subjected to chronic partial constriction of the sciatic nerve.

Materials and Methods: Six groups; each of 10 mice as follows: Oral saline treated control (group I), sham operated (group II), groups subjected to partial constriction of left sciatic nerve (groups III-VI); oral saline control group, groups treated with oral verapamil 15, 30 and 60 mg/kg respectively. Time course behavioral tests were observed namely noxious thermal and non noxious response as well as cold allodynia response at 0, 1,7,14 and 21 day of study. Serum visfatin and serum leptin levels, nerve reduced glutathione, spinal cord brain derived neurotrophic factor as end point biochemical measurements were assessed. All were done after three weeks of oral treatment.

**Corresponding author: Email: inasdarwish@gmail.com;*

Results: The study revealed significant antinociceptive effect of verapamil (*P*<0.001) with replenishment of reduced glutathione *(P*<0.01), significant increase in spinal cord levels of brain derived neurotrophic factor (*P*<0.001) and reduction of mean serum visfatin levels. No significant effect on mean serum leptin in any treated groups was observed.
Conclusions: Verapamil 60 mg, 30 mg/kg oral dose had the highest significant antinociceptive, regenerative and as well as ability to reduce serum visfatin level after three week therapy.

Keywords: Verapamil; visfatin; leptin; brain derived neurotrophic factor.

ABBREVIATIONS

Brain derived neurotrophic factor (BDNF); reduced glutathione (GSH).

1. INTRODUCTION

Nerve damage etiologies including avulsion, stretch, compression and contusion are frequently seen clinically as in obstetric or orthopedic traumas. These lesions lead to important functional impairments in most cases; therefore experimental models of nerve injuries have been developed over the last years in an attempt to mimic the clinical situation and to provide insights into new strategies that can improve nerve regeneration and functional outcomes. Neurapraxia is a reversible state where there is no anatomical rupture in the injured neuron is one example where function is completely recovered with no surgical interference. Axonotmesis is another example that occurs when there is a complete interruption of the axons, but the nerve supportive connective tissue is preserved [1,2]. A cascade of cellular and molecular events, described as Wallerian degeneration occurs throughout the distal stump. This process involves several phases in which the distal stump degenerates; the myelin associated with degenerating axons are degraded and removed by Schwann cells (SC), and blood-recruited macrophages. Injured axon regeneration following trauma depends on a delicate balance between growth-promoting and growth-inhibiting factors. Several neurotrophic factors, pro inflammatory cytokines, extracellular matrix molecules and hormones are secreted by neurons, SC, macrophages, the target tissue and cells present in the injury site, promoting neuronal survival [3,4]. Brain derived neurotrophic factor (BDNF) is a member of the neurotrophin family with an important role in the maintenance and formation of neuronal synapses, favoring a pivotal role in neuroprotection. However, BDNF is considered a crucial signaling molecule between microglia and neurons in neuropathic pain [5]. Ca ions are regulators of many metabolic pathways serving as second messenger. Moreover, Ca ion influx into presynaptic terminal is important for neurotransmission .Indeed, the transection of axons initiates a large depolarizing voltage discharge that travels back to the soma and triggers vigorous spiking activity and sustained depolarization [6]. This extensive electrical activity produces a strong calcium influx in both the axon and the soma. Propagation of this response requires the activation of voltage-gated sodium channels and is necessary for regeneration. Therefore stimulating the endogenous anti-inflammatory reaction and decreasing pro-inflammatory processes by a channel blocker might have a role in pain modulation or regeneration. Among the calcium channel blockers is verapamil that had been used in many cardiovascular diseases as hypertension, arrhythmias and had been tried as an antinociceptive [7]. In addition, verapamil had been suggested as a neuroprotective to prevent the behavioral deficits in an experimental Alzheimer's that might be due to the regulation of calcium homeostasis [8]. Non calcium dependent mechanism as in inflammation related neurodegenerative diseases was newly demonstrated [9].

Recently, the role played by adiponectins in mediating degenerative diseases has been postulated. Adiponectins as leptin and visfatin are proteins primarily secreted by adipocytes. Beside their typical role in obesity; they exert widespread action in stimulating cytokines expression that has pathogenic implications in musculoskeletal disorders, documented in degenerative joint disease. Leptin has been reported to play a significant role in thermo genesis, synaptic plasticity, and it has been shown to exert neuroprotective effects in ischemia, Parkinson’s and Alzheimer’s diseases [10-12].

Visfatin, the second adiponectin, known as pre-B-cell colony-enhancing factor (PBEF) or nicotinamide phosphoribosyltransferase (NAMP), acts as an insulin analogue on the insulin receptor to exert mimicking insulin actions .Its concentrations have been shown to be elevated in many conditions as human obesity with proved pro inflammatory and immune modulating properties [11-14]. Whether leptin and visfatin are pro inflammatory, pain related proteins or not, is still a matter of debate. Chronic Sciatic nerve constriction (CSC) or injury, induces painful neuropathy is an employed model for induction of neuropathic pain in experimental animals [15].

The current study was designed to compare three dose levels of verapamil on induced constriction sciatic nerve injury in mice and to assess the role played by leptin or visfatin in mediating regeneration.

2. MATERIALS AND METHODS

2.1 Experimental Animals

Mice were housed in cages of four and exposed to 12- h day/night cycles with free access to food and water (Pharmacology Department; Faculty of Medicine; Alexandria University, Egypt). Mice weighed from 20-25 g, and aged from 11-12 weeks when the study began. All animal care and experiments were carried out according to standard international guidelines protocols, approved by the Alexandria Faculty of Medicine Ethical Committee (AFMEC), Alexandria. All behavioral tests were assessed from 9:00 am to 5:00 pm in a temperature controlled room. Efforts were made to minimize animal numbers and suffering.

2.2 Experimental Groups

After two weeks habituation to experiment environment, 60 mice were randomly classified to six groups each of 10:

- **Group I:** normal control mice injected by a single i.p. injection of normal saline and received 2 ml saline orally for three weeks, starting one week following random grouping.
- **Group II:** normal control mice: sham operated injected by a single i.p. injection of thiopental sodium (35 mg/kg), and the sciatic nerve was exposed without any ligation. This group received 2 ml saline p.o. for three weeks, starting from day 1 after being sham operated.
- **Group III:** Injured control Mice: Received 2ml saline p.o daily from day 1 up to three weeks after surgery for chronic sciatic nerve constriction (CSC) [15,16].
- **Group IV:** mice treated orally with verapamil (Sigma –Fluka Chemical Co., St. Louis, MO) in a dose of 15 mg/kg in saline for three weeks, starting from day 1 following CSC [7].
- **Group V:** mice treated orally with verapamil 30 mg/kg in saline for three weeks, starting from day 1 following CSC.
- **Group VI:** mice treated orally with verapamil 60 mg/kg in saline for three weeks, starting from day 1 following CSC.

Dosing selection of verapamil was detected after testing percentage of acetic acid (3% v/v in saline) induced writhes reduction in mice along 15 min after another 15 min after i.p. injection of 5, 10, 20, 40, 60 mg /kg of verapamil dissolved in saline (0.1 ml/10 g) compared to saline control groups [7].

2.3 Model Preparation; Chronic Constriction on Sciatic Nerve

The mice were anesthetized as mentioned before. The hair of the mice's lower back in thigh region of left paw was shaved, and the skin was sterilized with povidone-iodine. Then the skin was incised and a cut was made directly through the biceps femoris muscles to expose the sciatic nerve. Two ligatures (silk 4-0), were placed around the nerve proximal part of the trifurcation with a distance of 1 mm between each ligature. The ligatures were loosely tied until a short flick of the left hind limb was observed. Muscular and skin layer was immediately sutured with thread and topical antibiotic was applied at once [16]. Nociceptive threshold was assessed before, to exclude non responsive mice, and after performing surgery on different days; 0, 7, 14, and 21st day.

2.4 Specimen Preparation

After 24 hours of assessing the last behavioral tests; after 3 weeks study period, the animals were exposed to light ether anesthesia, then sacrificed, blood was collected from abdominal aorta and allowed to clot at 25°C. The blood was centrifuged at 3000 rpm from 15 min at 4°C and

serum was divided into 2 aliquots, kept at -20°C for determination of serum leptin and visfatin. Whereas left sciatic nerve and surrounding muscle were excised, separation of nerve and muscle tissue were done, and then preserved separately at -20°C for later determination of reduced glutathione. Lumbar vertebrae (from L2) to sacral vertebrae were separated, stored at liquid nitrogen for subsequent determination of spinal brain derived neurotrophic factor

2.5 Behavioral Parameters

2.5.1 Thermal hyperalgesia

By hot plate method that was maintained at 53± 0.5°C with cutoff time of 15 s, latency to hind paw licking or vertical jumping was determined. Mean of three times response was calculated [17] .

2.5.2 Static mechanical hyperalgesia

The mouse's left hind paw was placed on the pressure applicator, and then exposed to a steadily increased pressure on the dorsal surface of the paw. The force in grams needed to induce paw withdrawal was regarded as the pain threshold. Cutoff force was 250 gm [16,17].

2.5.3 Cold chemical allodynia

The mice were placed on the top of a wire mesh grid, allowing access to the hind paws. Acetone (0.1 ml) was sprayed on the plantar surface of left hind paw of mouse. Cold chemical sensitive reaction with respect to either paw licking, shaking or rubbing the left hind paw was observed and recorded as paw lifting duration for 20 s test period [18].

2.6 Biochemical Measurement

2.6.1 Reduced Glutathione in sciatic nerve (GSH)

This method is based on the development of a yellow color when n5, 5\ dithio-2 nitrobenzoic acid (DTNB) is added to a sulfhydryl compounds. The stable color was read at 412 nm. Standard curve was constructed using serial dilutions of stock GSH standard.GSH level was expressed as nmol/g tissue [19].

2.6.2 Spinal BDNF assay

Stored spinal cord samples, were then homogenized in extraction solution (100 mg/ml); containing 0.4 M Nacl, 0.05% tween 20,0.5% bovine serum albumin, 0.1 mM phenylmethylsulphonyl Fluoride, 0.1 mM benzytonium chloride,10 mM EDTA, 20 KIU aprotinin prepared in phosphate buffered saline (PBS). The homogenates were centrifuged at 10,000 rpm for 10 min at 4°C; the supernatants were kept at -20°C. The spinal BDNF concentrations were determined at 1:3 dilution in PBS contains 0.1% BSA, using an ELISA kit in accordance with the manufacturer instructions (Boster biological technology co., Ltd, Pleasanton, CA). The concentrations were calculated using a standard curve, expressed as pg/ml [20].

2.6.3 Seum leptin

Samples were assayed using the DRG leptin Elisa Kit (Germany). It is based on the sandwich technique. Levels were expressed in ng/ml [21].

2.6.4 Serum visfatin

Samples were assayed using the rat visfatin enzyme immunoassay Kit provided by the Ray Biotech, Inc. (USA). It is based on the principle of competitive enzyme immunoassay. Levels of serum visfatin were expressed in ng/ml [22].

2.7 Statistical Analysis

Statistical Package for Social Science SPSS 20.0 soft ware was used for data analysis. Results were expressed as a mean ± standard error of the mean (SEM) through ANOVA test. A post hoc test was determined with Least Significant Difference (LSD).

3. RESULTS AND DISCUSSION

3.1 Behavioral Study

Time course measurements for hot plate latency, paw withdrawal force and latency to cold chemical allodynia at 0,7,14, and 21days revealed the significant antinociceptive effect of treated groups compared to control groups. No significant difference was detected between mean latency time to hot plate and paw withdrawal force at 14^{th} and 21^{st} days for each group (P =0.486, 0.577 respectively). All treated groups had significant change compared to normal control groups regarding *static mechanical hyperalgesia test*, but not revealed any significant difference in paw withdrawal force compared to CSC group. *For both thermal*

hyperalgesia and cold allodynia tests; verapamil, in a dose of 60 mg/kg (group VI) had significant difference compared to lowest dose; 15 mg/kg (group IV) have shown statistical significant difference compared (Table 1, Fig. 1).

3.2 Biochemical Measurements

Spinal brain derived neurotrophic factor mean spinal cord (BDNF) level in CSC was significantly reduced compared to control groups. There is statistical significant increase in all treated groups (groups IV, V and VI) compared to CSC non treated group ($p < 0.001$). No significant difference was accomplished in between higher verapamil doses (groups V and VI), $p=0.66$. There is statistical significant difference between the highest and lowest doses of verapamil (VI and IV). The mean sciatic nerve reduced glutathione levels in CSC was significantly reduced compared to control groups. There is statistical significant increase in all treated groups compared to CSC non treated group ($p < 0.01$). No significant difference in between three verapamil doses (groups IV, V and VI). Mean serum leptin levels showed no statistically increase in verapamil treated group groups .Mean serum visfatin levels revealed statistical significant increase in CSC injured control group compared to both control groups. Both verapamil groups V and VI treated groups revealed significant lowered mean serum visfatin levels compared to CSC group (Fig. 2)

3.3 Discussion

The primary objective of the current experimental study was to test role of adiponectins in CSC and to assess the possible neuroprotective role of verapamil on behavioral responses to noxious and non-noxious pain stimuli and its reflected impact on brain derived neurotrophic factor concentration. Different tested doses of verapamil were compared in the present study, which to our knowledge was not used before in this model. The statistically significant improvement at day 14^{th} and 21^{st} in all groups; reflects that functional sciatic nerve regeneration in treated groups that had begun at least after 2weeks duration following left CSC. Partial constriction interrupts all axons but Schwann cell basal laminae are preserved so that regeneration is optimal [23-25]. Field et al. [26]; suggested that thermal hyperalgesia, static and dynamic allodynia were respectively signaled by C-, Aδ- and Aβ/ capsaicin insensitive Aδ- primary sensory neurons. They further concluded that pregabalin possesses a superior antiallodynic profile than morphine and may represent a novel class of therapeutic agents for the treatment of neuropathic pain. The spinal posterior ganglion neurons have various types of voltage-dependent calcium channels because the units of alpha-1 of these channels are the main determiners of the pharmacologic and biophysical features, whereas sub-units of alpha-2, sigma-1 and gamma along with alpha-1 are recognized as modifiers of the channel performance [6]. The findings of this study show that oral administration of verapamil in a dose-dependent manner reduced the symptoms of neuropathic pains, including latency to hot plate noxious algesia, mechanical pressure and cold chemical allodynia. Verapamil is a differential blocker of L-type calcium channel, basically doesn't affect peripheral nerve transmission. Most of experimental findings showed the role played by N calcium channels in the emergence of symptoms of neuropathic pain. Although, the activity and expression of type L calcium channels varies during neuropathic pain, the role of these channels in the emergence of neuropathic pain symptoms is not clear [27].

The current study revealed that verapamil effect in increasing nerve reduced glutathione level compared to CSC injury control group. Our findings partially match the neuroprotective, anti-inflammatory findings to calcium inhibition in a constriction sciatic nerve model and other neurological models [28,8,9]. The dose-dependent effect on reduced glutathione levels might be correlated to verapamil effect on visfatin and BDNF findings. Verapamil at 15, 30, 60 mg/kg daily orally had significant effect on serum visfatin level, but no significant effect on serum leptin level. Muthuraman et al. [28]; proved the role of calcium inhibition and antioxidative and anti-inflammatory effect. With the experimental use of another calcium channel blocker, Selt et al. [29]; proved that one month treatment of lomerizine a voltage gated L and T type calcium channel blocker, protects retinal ganglion cells, and incompletely preserves visual function with limitation of elements of secondary degeneration, including macrophage infiltration. Visfatin is the most recently identified adipocytokine which appears to be preferentially produced by visceral adipose tissue and has insulin-mimetic actions. Visfatin expression is increased in animal models of obesity and its plasma concentrations are increased in humans with abdominal obesity or type 2 diabetes mellitus. Visfatin binds to the insulin receptor at a site distinct from insulin and

exerts hypoglycemic effect. Visfatin was also identified in inflammatory cells and its levels were increased in various inflammatory conditions. Other studies revealed correlations of visfatin levels with osteoarthritic pain [30,31]. Although leptin is not a classical cytokine, several immune cells including polymorphonuclear leukocytes, monocytes, macrophages and lymphocytes bear leptin receptors and their activity can be modulated by leptin. Most of leptin pro-inflammatory activities appear to be mediated by a long OBRb receptor. Leptin has certain structural similarities to classical cytokines like IL-6, GM-CSF or IL-12 [10,11]. Leptin levels in our study showed no significant rise in any of three doses of verapamil. The question is the crucial role of visfatin in such experimental or clinical studies, is it a cause or result for pain and subsequent healing? Further studies are needed to answer such a question.

Table 1. Mean values ± S.E.M. of behavioral tests for control and Verapamil treated groups at the 21st day of study after CSC induction in mice; n = 6

Tests groups	Latency (s) thermal hyperalgesia	Paw pressure (g) static hyperalgesia	Paw lifting(s) cold chemical allodynia
I Control	3.16±0.65	35.33±1.99	1.50±0.22
II Sham operated	2.83±0.40	34.00±3.17	1.50±0.22
III CSC control	11.33±0.67[a]	21.83±3.98 [a]	9.0±0.25[a]
IV Verapamil+CSC (15 mg/kg)	5.17±0.48[ab]	25.50±0.43 [a]	5.50±0.43[a b]
V Verapamil+CSC (30 mg/kg)	6.67±0.71[ab c]	25.67±0.84[a]	3.83±0.3 [a b]
VI Verapamil+CSC (60 mg/kg)	5.33±0.42[a b]	25.17±1.50[a]	3.67±0.56[a b c]

n= number of studied mice in each group [a] < 0.001 significant from control groups b< 0.01 significant from CSC control group. [c] =0.05 significant from group IV

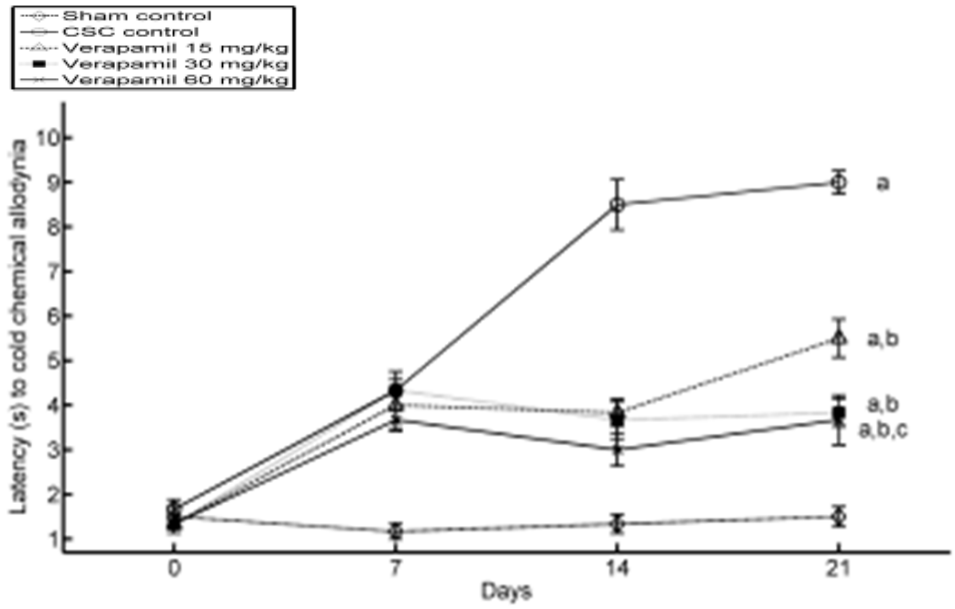

Fig. 1. Time course study for latency (s) to cold chemical allodynia. results are (Mean±S.E.M) of verapamil treated groups , sham operated and CSC control groups at, 0,7,14, 21st day post chronic sciatic nerve constriction (CSC) in mice. n= 6 mice in each group. [a] = 0.05 significant from sham operated. [b] < 0.05 significant from CSC control. [c] < 0.05 significant from verapamil 15 mg/kg

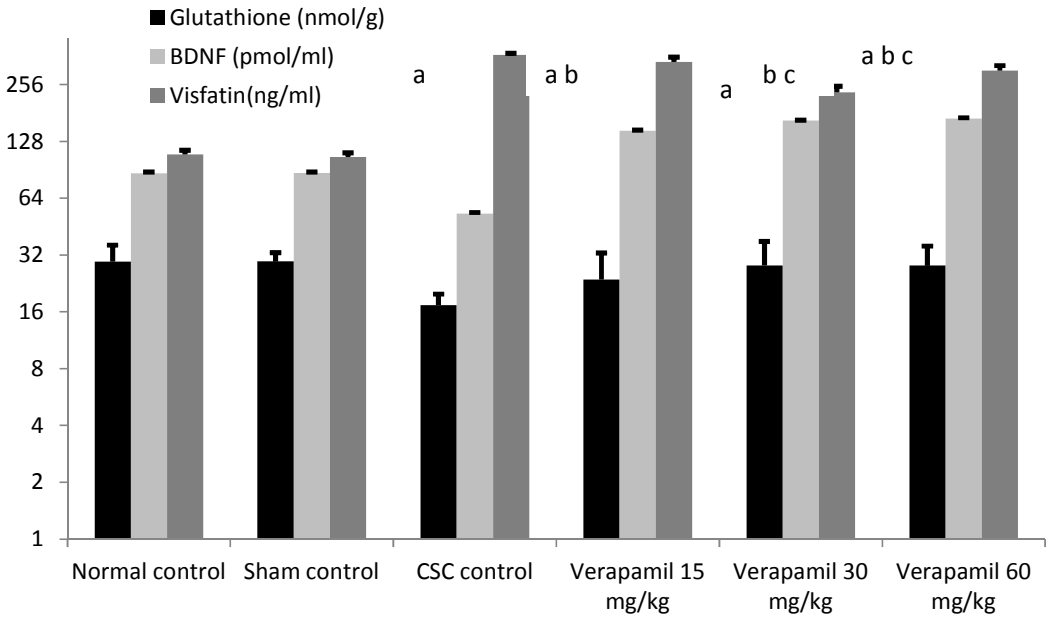

Fig. 2. Mean values ± S.E.M. of biochemical measurements for control and verapamil treated groups at the 21st day of study after CSC induction in mice .n= number of studied mice (6-7) in each group. a < 0.001 significant from Control groups. b < 0.01 significant from CSC control group. c =0.05 significant from Verapamil 15 mg/kg group

4. CONCLUSION

The present study demonstrated that verapamil has a neuroprotective effect, evidenced by an increase in BDNF. Its neuroprotective effect might be due to its observed anti-oxidant effect as well as its significant effect on visfatin level.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Sulaiman W, Dreesen TD. Effect of local application of transforming growth factor-β at the nerve repair site following chronic axotomy and denervation on the expression of regeneration-associated genes. Laboratory investigation. J Neurosurg. 2014;121(4):859-74.
2. Chierzi S, Ratto GM, Verma P, Fawcett JW. The ability of axons to regenerate their growth cones depends on axonal type and age, and is regulated by calcium, cAMP and ERK. Eur J Neurosci. 2005;21:2051–2062. [PubMed: 15869501
3. Fregnan F, Muratori L, Simões AR, Giacobini-Robecchi MG, Raimondo S. Role of inflammatory cytokines in peripheral nerve injury. Neural Regeneration Research. 2012; 7(29):2259-2266.
DOI:10.3969/j.issn.1673-5374.2012.29.003.
4. Jeon SM, Lee KM, Cho HJ. Expression of monocyte chemoattractant protein-1 in rat dorsal root ganglia and spinal cord in experimental models of neuropathic pain. Brain Res 2009;1251:103-11.
DOI: 10.1016/j.brainres. 2008.11.046. Epub 2008 Nov 27.
5. Coull JA, Beggs S, Boudreau D, Boivin D, Tsuda M, Inoue K, Gravel C, Salter WN, De Koninck Y. BDNF from microglia causes the shift in neuronal anion gradient

underlying neuropathic pain. Nature. 2005; 438:1017–1021.
6. Yaksh, Tony L. Calcium channels as therapeutic targets in neuropathic pain. The Journal of Pain. 2006;7.1:S13-S30.
7. Koleva M, Dimova S. Effects of nifedipine, verapamil, diltiazem and trifluoperazine on the antinociceptive activity of acetaminophen. Methods Find Exp Clin Pharmacol. 2000;22:741–745.
8. Popović M, Caballero-Bleda M, Popović N, Bokonjić D, Dobrić S.Neuroprotective effect of chronic verapamil treatment on cognitive and noncognitive deficits in an experimental Alzheimer's disease in rats. Int J Neurosci. 1997;92(1-2):79-93.
9. Liu Y, Lo YC, Qian L, Crews FT, Wilson B, Chen HL, Wu HM, Chen SH, Wei K, Lu RB, Ali S, Hong JS. Verapamil protects dopaminergic neuron damage through a novel anti-inflammatory mechanism by inhibition of microglial activation. Neuropharmacology. 2011;60(2-3):373-80.
10. Lago F, Dieguez C, Gomez-Reino J, Guallilo O. Adipokines as emerging mediators of immune response and inflammation. Nat Clin Pract Rheumatol. 2007;3:716–24.
11. Zhang F, Wang S, Signore AP, Chen J. Neuroprotective effects of leptin against ischemic injury induced by oxygen-glucose deprivation and transient cerebral ischemia. Stroke, 2007;38:2329–2336.
12. Guo Z, Jiang H, Xu X, Duan W, Mattson MP. Leptin-mediated cell survival signaling in hippocampal neurons mediated by JAK STAT3 and mitochondrial stabilization. J Biol Chem. 2008;283:1754–1763.
13. Chen MP, Chung FM, Chang DM, Tsai JC, Huang HF, Shin SJ. Elevated plasma level of visfatin/pre-B cell colony enhancing factor in patients with type 2 diabetes mellitus. J Clin Endocrinol Metab. 2006; 91:295–299.
14. Moschen AR, Kaser A, Enrich B, Mosheimer B, Theurl M, Niederegger H, Tilg H .Visfatin, an adipocytokine with proinflammatory and immunomodulating properties. J Immunol. 2007;178:1748–1758.
15. Lee SH, Won R, Baik E J, Lee SH, Moon C H. An Animal model of neuropathic pain employing injury to sciatic nerve branches. Neuroreport. 2000;11:657-661.
16. Kiguchi N, Maeda T, kobayashi Y, Fukazawa Y, kishioka S. Activation of extracellular signal–regulated kinase in sciatic nerve contributes to neuropathic pain after partial sciatic nerve ligation in mice. Aneth Analg. 2009;109:1305-11.
17. Le Bars D, Gozariu M, Cadden SW.Animal models of nociception. Pharmacol Rev. 2001;53:597-652.
18. Chol Y, Yonn YW, Na SH, Kim SH, Chung JM. Behavioral signs of ongoing pain and cold allodynia in rat model of neuropathic pain. Pain. 1994;59:369-376.
19. Ellman GL. Tissue sulphydryl groups. Arch Biochem Biophy1959; 82:70–7.
20. Kovalchuk Y, Hanse E, Kafitz KW, Konnerth A. Postsynaptic induction of BDNF-mediated long-term potentiation. Science. 2002;295:1729-1734.
21. Haider DG, Schaller G, Kapiotis S, Maier C, Luger A, Wolzt M. The release of the adipocytokine visfatin is regulated by glucose and insulin. Diabetologia. 2006; 49:1909-1914.
22. Duan Y, Hao D, Li M, Wu Z, Li D, Yang X, Qiu G. Increased synovial fluid visfatin is positively linked to cartilage degradation biomarkers in osteoarthritis. Rheumatol Int. 2012;32:985–90.
23. Newton RA, Bingham S, Case PC, Sanger GJ, Lawson SN. Dorsal root ganglion neurons show increased expression of the calcium channel α2δ-1 subunit following partial sciatic nerve injury. Mol Brain Res 2001;95:1–8.
24. Ma W, Quirion R. Partial sciatic nerve ligation induces increase in the phosphorylation of extracellular signal-regulated kinase (ERK) and c-Jun N-terminal kinase (JNK) in astrocytes in the lumbar spinal dorsal horn and the gracile nucleus. Pain. 2002;99:175–184.
25. Narciso MS, Mietto Bds S, Marques SA, Soares CP, Mermelstein C dos S, El-cheikh MC, Martinez AM. Sciatic nerve regeneration is accelerated in galectin-3 knockout mice. Exp Neurol. 2009;217:7–15.
26. Field MJ, Bramwell S, Hughes J, Singh L. Detection of static and dynamic components of mechanical allodynia in rat models of neuropathic pain: Are they signaled by distinct primary sensory neurons? Pain. 1999;83:301-11.
27. Dobremez E, Bouali-Benazzouz R, Fossat P, Monteils L, Dulluc J, Nagy F, Landry M. Distribution and regulation of L-type calcium channels in deep dorsal horn neurons after sciatic nerve injury in rats. Eur J Neurosci. 2005;21:3321-3333.

28. Muthuraman A, Singh N. Attenuating effect of *Acorus calamus* extract in chronic constriction injury induced neuropathic pain in rats: An evidence of anti-oxidative, anti-inflammatory, neuroprotective and calcium inhibitory effects. BMC Complementary and Alternative Medicine. 2011;11:24.
29. Selt M, Bartlett CA, Harvey AR, Dunlop SA, Fitzgerald M. Limited restoration of visual function after partial optic nerve injury; a time course study using the calcium channel blocker lomerizine. Brain Res Bull. 2010;16:461-71.
30. Rechardt M, Shiri R, Lindholm H, Lindholm H, Karppinen J, Viikari-Juntura E. Associations of metabolic factors and adipokines with pain in incipient upper extremity soft tissue disorders: A cross-sectional study. BMJ Open. 2013; 3:e003036. DOI:10.1136/bmjopen-2013-003036
31. Harvey VL, Dickenson AH. Behavioural and electrophysiological characterization of experimentally induced osteoarthritis and neuropathy in C57Bl/6 mice. Molecular Pain. 2009;5:18. DOI:10.1186/1744-8069-5-18.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=1121&id=12&aid=9441