ACTA SCIENTIFIC NEUROLOGY

Volume 2 Issue 7 July 2019

Research Article

Papain, an active constituent of *Carica Papaya* ameliorates neuropathic pain in rats subjected to sciatic nerve ligation by mitigating oxidative damage and excitotoxicity

Lavanya Yaidikar[1]*, Swapna Pokkula[2], Prathap Naik[1], Santhrani Thakur[3], M Niranjan Babu[1]

[1]*Seven Hills College of Pharmacy, Venkatramapuram, Tirupati, Andhra Pradesh, India*

[2]*Max Institute of Pharmaceutical Sciences, Velugumatla, Khammam, Telangana, India*

[3]*Sri Padmavathi Mahila Visvavidyalayam, Tirupati, Andhra Pradesh, India*

***Corresponding Author:** Dr. Lavanya Yaidikar, Associate Professor, Department of Pharmacology, Seven Hills College of Pharmacy, Tirupati, Andhra Pradesh, India.

Received: April 17, 2019; **Published:** June 04, 2019

Abstract

Neuropathic pain (NP) is a disability disorder mainly affects one's quality of life. Excitotoxicity due to Sodium channel opening, oxidative stress and inflammation has been implicated in the pathogenesis of NP. *Carica papaya*, a neutraceutical with wide range of medicinal properties. Papain (PN) is the active constituent of *Carica papaya* with potent antioxidant and anti-inflammatory properties. Hence, we aimed to evaluate the protective role of PN in sciatic nerve ligation (SNL) induced neuropathic pain in rats. We assessed a battery of behavioral and biochemical changes after SNL in rats. Briefly, male wistar rats were divided into four groups of eight rats in each group; Sham control, SNL control, PN 50 mg/kg/p.o and PN 100 mg/kg/p.o treated groups. The drug or vehicle were administered orally by gavage once daily for 14 days. Nociceptive threshold was assessed on 15th day using a battery of tests such as foot deformity score, cold allodynia, motor coordination test, spontaneous locomotor activity and transfer latency time. Biochemical markers namely glutamate, calcium, tissue protein, lipid peroxides, reduced glutathione, and superoxide dismutase levels were measured in sciatic nerve homogenate. PN 100 mg/kg significantly ($p<0.001$) attenuated the SNL induced nociceptive threshold, oxidative stress and excitotoxicity changes which was supported by histological studies in sciatic nerve. These findings suggested that PN attenuated neuropathic pain in rats through mitigating oxidative stress and excitotoxicity.

Keywords: Neuropathic Pain; Oxidative Damage; Excitotoxicity; Papain; *Carica Papaya*

Introduction

Neuropathic pain (NP) is considered as one of the neurological disorder due to dysfunction of peripheral or central nervous system characterized by allodynia, hyperalgesia and persistant pain [1,2]. The management of neuropathic pain is extremely difficult due to complexity in its pathophysiology, which is unclear [3,4]. Many researchers reported that NP is due to activation of immune cells, release of proinflammatory cytokines such as tumor necrosis factor – α (TNF- α), Interleukin 1β (IL-1β), Interleukin-6 (IL-6) [5,6], opening of calcium channels [7,8], sodium channels opening [9], oxidative stress (overwhelming of free radicals) [6,10]. This implies that multiple cascade events occur in NP, which is challenging to manage. Largely, neuropathic pain affects the quality of life. The currently available allopathic drugs or treatments provide symptomatic relief from the pain and are associated with severe adverse effects. Therefore, it is necessary to search for novel alternative therapeutic strategies from herbal source for neuropathic pain [11,12].

Now-a-days, various medicinal plants and their compounds have been reported effective for the management of neuropathic pain. This indicates the importance of Indian system of Medicine. Isolated plant compounds [11] such as α-β- Amyrin, β- Caryophyllene, Cannabidiol, Genistein, Hhesperitin, Kaempferol, Lappaconitine, Linalool, Liquiritigenin, Luteolin, Mangiferin, Naringin, Rutin, Quercetin, Silibinin, Oxymatrine, Triptolide, Verbacoside,

Tormentic acid were scientifically reported their effectiveness against neuropathic pain through mitigation of various pathways such as inflammation, oxidative stress, inhibiting sodium channels, inhibiting calcium channels, inhibiting glutaminergic system. Proteolytic enzymes from medicinal plants such as bromelain, papain, pancreatin, trypsin, chymotrypsin, rutin play an essential role in inflammation and other functions of the immune system [13,14]. They can degrade pathogenic complexes, which are the principal causes of certain kidney diseases, nerve inflammation, rheumatoid arthritis. They can modulate the inflammatory process through different mechanisms. There is no scientific report of papain against neuropathic pain. Hence, we made an attempt to evaluate the efficacy of papain (PN) against partial sciatic nerve ligation induced neuropathic pain in rats.

Material and Methods

Drugs and chemicals

Papain (PN) was obtained as a free gift sample from Enzyme Biosciences, Gujarat, India. All the chemicals used were of analytical grade and were procured from Merck or Sigma or S.D. Fine Chemicals.

Animals

Swiss Albino male rats of weighing approximately 120–130 g were used for the study. Animals were acclimatized under laboratory condition for 2 weeks prior to the experiments. They were maintained under standard conditions of temperature (23 ± 2°C) and humidity (50 ± 10%) with an alternating 12 h light/dark cycles. The animals had free access to tap water and fed standard pellet diet (Agro Corporation Private Ltd., Bangalore, India).

Surgical procedure

The rats were subjected to SNL surgery under general anesthesia with intramuscular injection of ketamine 100 mg / kg and 10 mg /kg xylazine. Briefly, the rats were placed in prone position, hair was shaved from the hind limb and mid back. The sciatic nerve was exposed at the dorsa caudal region. An incision was made starting 0.5 cm laterally from the animal's midline and extended laterally for 3cm towards the tibio femoral articulation. The femoral biceps and gluteus muscles were separated using blunt dissection forceps to allow access to provide exposure to sciatic nerve and half of the left sciatic nerve was ligated at the upper thigh level using an 8-0 nylon suture. Sham surgery was done by exposing the sciatic nerve without ligation [15]. Nociceptive threshold was assessed on 15th day. Then the animals were sacrificed by cervical decapitation, sciatic nerve was collected, homogenized and supernatant was used for biochemical studies.

Experimental protocol

Forty rats were randomly divided into five groups of six animals in each group and they were treated as follows.

- Group 1 (Sham control group) – animals received vehicle only.
- Group 2 (Sciatic nerve ligation group - SNL) - animals received vehicle only.
- Group 3 (PN pre-treatment group) – received PN (50 mg/kg) orally by gavage once daily for 14 days prior to SNL surgery.
- Group 4 (PN pre-treatment group) – received PN (100 mg/kg) orally by gavage once daily for 14 days after SNL surgery.

Pain threshold assessment

Foot deformity score

The foot deformation in ligated and drug treated groups were assessed by deformation score. The rat was placed on a plate with a neutral temperature and the posture of the foot was observed. The foot deformation was scored as follows: [16]

- Score 0 if the paw is in normal position with fanned toes.
- Score 1 if the toe is ventroflexed.
- Score 2 if the paw is everted so that only the internal edge of the paw touches the floor.

Cold allodynia test

Cold allodynia test was performed as per the method described by Naik., *et al*. [17]. In this method, the left hind paw of the rat was gently submerged in ice cold water (4 ± 1^{0}C) in a beaker. The paw withdrawal latency was observed with a maximum cutoff time of 20 s.

Motor co-ordination test

Motor co-ordination was evaluated by a rota rod device as described by Jones and Roberts [18]. Briefly, rats were placed individually for one minute on the rotating rod (25 rpm). The time taken by the rat to fall off from the rod during one minute was recorded.

Test for spontaneous locomotor activity

Photoactometer test was employed to assess the effect of drug treatment on spontaneous motor activity by using actophotometer. Each animal was observed for a period of 5 min in a square closed field area (30×30×30 cm) equipped with 6 photocells in the outer wall. Interruptions of photocell beam were recorded by digital counter [19].

Transfer latency test by elevated plus maze

The Elevated plus maze consists of two open and two enclosed arms. The animals were placed individually at the end of either of the open arm and the time by the animals to move from open to enclosed arm (transfer latency) was noted on the first day. The transfer latency is again recorded 24hrs after first exposure. The transfer latency on the first day trial serves as acquisition (learning) and the retention consolidation (memory) is examined 24hrs later. Each animal was used only once. One hour administration of the drug the test was carried out and the memory was assessed.

Biochemical analysis

After 14 days of surgery, animals were sacrificed by cervical dislocation and sciatic nerve was immediately isolated, homogenized with 0.1 M Tris–HCl buffer (pH 7.4,) and supernatant of homogenate was employed to estimate the levels of total protein content, lipid peroxides, reduced glutathione (GSH), Superoxide dismutase (SOD) were measured.

Estimation of glutamate

Glutamate level was measured according to the method described by Bernt and Bergmeyer [20], with minor modifications. To 1 ml of supernatant, 2 ml of perchloric acid was added and pH was adjusted to 9.0 with phosphate buffer. The resulting mixture was subjected to centrifugation at 1500×g for 15 min and was allowed to stand for 10 min in an ice bath and then filtered through fluted filter paper. Absorbance was measured at 340nm. The glutamate level was expressed as µmol/g tissue.

Estimation of total calcium

Total calcium levels were estimated using commercially available kits (Span diagnostics, India).

Estimation of tissue protein

Tissue protein content was estimated in the sciatic nerve according to the method of Lowry *et al*. [21], using BSA (bovine serum albumin) as a standard.

Estimation of lipid peroxides

Estimation of lipid peroxides was done by measuring the levels of malondialdehyde (MDA) [22]. The concentration of MDA in sciatic nerve homogenates was expressed in terms of nM MDA/mg protein.

Estimation of reduced glutathione levels

GSH was measured according to the method of Ellman [23]. Equal quantity of sciatic nerve homogenate was mixed with 10% trichloroacetic acid and centrifuged to separate proteins. To 0.01 ml of this supernatant, 2 ml of phosphate buffer (pH 8.4), 0.5 ml of 5,5′-dithio, bis (2-nitrobenzoic acid) and 0.4 ml double-distilled water was added. Mixture was vortexed and the absorbance was measured at 412 nm within 15 min. The concentration of reduced glutathione was expressed as µg/mg of protein.

Estimation of superoxide dismutase levels

SOD activity was measured according to the method of Misra and Fridovich, [24] by monitoring the auto-oxidation of (-) – epinephrine at pH 10.4 for 4 min at 480 nm. Briefly, 100 µl of supernatant was added to 880 µl of 0.05 M carbonate buffer containing 0.1 mM disodium edentate (pH 10.4), and 20 µl of 30 mM epinephrine (in 0.05% acetic acid) was added to the mixture and the optical density values were measured at 480 nm for 4 min on a UV-Visible Spectrophotometer, activity was expressed as the amount of enzyme that inhibits the oxidation of epinephrine by 50% which is equal to 1 unit. The SOD activity is expressed as U/mg protein.

Histopathology

Samples of sciatic nerve were kept in the fixative solution (10% formalin) and cut into 4-µm thickness. Staining was done by using hematoxylin and eosin as described by Yukari., *et al*. [25]. Nerve sections were analyzed qualitatively under light microscope for axonal degeneration.

Statistical analysis

All the results were expressed as mean ± standard error of mean (S.E.M). Data was analyzed using one-way ANOVA followed by post hoc analysis of Dunnetts test. A value of $p < 0.05$ was considered to be statistically significant.

Results

Effect of PN on foot deformity

SNL induced foot deformity in all animals. The rats with induced pain developed abnormal gait and posture. The foot was ventro-

flexed, with the toes held tightly together and rats were unwilling to place weight on the foot of the injured side. Foot positioning and toe spread rating was significantly (p<0.001) different between control and experimental groups. PN 50 and 100 mg/kg pre-treated rats significantly (p<0.001) prevented the SNL induced foot deformity when compared with SNL control group (Figure 1).

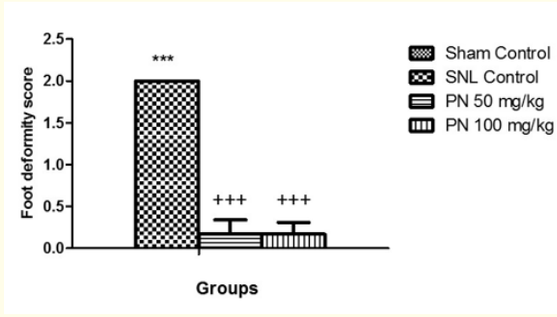

Figure 1: Effect of PN on Foot deformity score.
Values are expressed as mean ± standard error of mean (SEM, n=6).
Analyzed by one-way ANOVA followed by post hoc Dunnetts test.
***(p<0.001) vs sham control group.
+++(p<0.001) vs SNL control group.

Effect of PN on motor co-ordination

SNL induced significantly (p<0.001) decreased motor performance when compared with sham control group. PN 50 and 100 mg/kg pretreated groups significantly (p<0.01) prevented the SNL induced decrease in motor performance when compared with SNL control group (Figure 3).

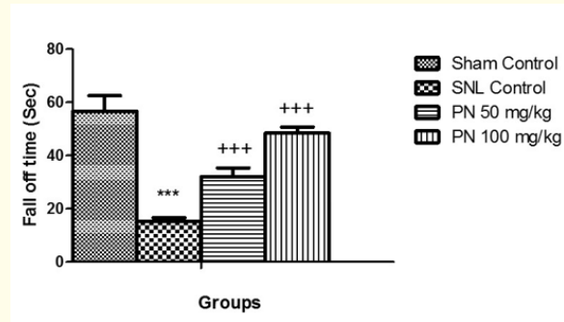

Figure 3: Effect of PN on motor coordination.
Values are expressed as mean ± standard error of mean (SEM, n=6).
Analyzed by one-way ANOVA followed by post hoc Dunnetts test.
*(p<0.05), ***(p<0.001) vs sham control group.
+++(p<0.001) vs SNL control group.

Effect of PN on cold allodyna

SNL group showed significantly (p<0.001) tail withdrawl time when compared with sham control group. However PN 50 and 100 mg/kg pre-treated rats significantly (p<0.001) prevented the SNL induced increased tail withdrawl time when compared with SNL control group (Figure 2).

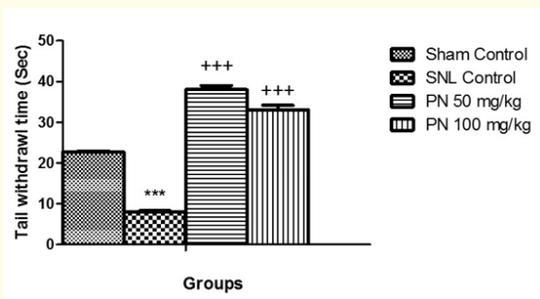

Figure 2: Effect of PN on cold allodynia.
Values are expressed as mean ± standard error of mean (SEM, n=6).
Analyzed by one-way ANOVA followed by post hoc Dunnetts test.
*(p<0.001), **(p<0.01), ***(p<0.001) vs sham control group.
+++(p<0.001) vs SNL control group.

Effect of PN on spontaneous locomotor activity

Spontaneous locomotor activity in SNL control group was significantly (p<0.001) decreased when compared with sham control group. The decrease in spontaneous locomotor activity was significantly (p<0.001) attenuated in PN 50 and 100 mg/kg pretreated groups (Figure 4).

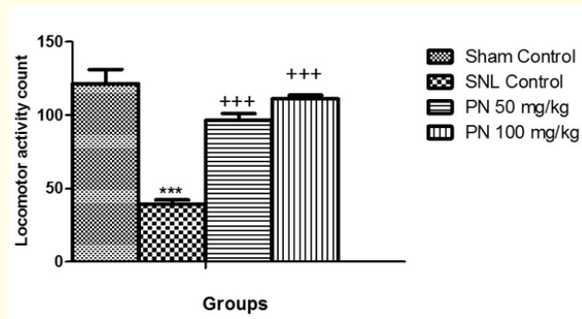

Figure 4: Effect of PN on spontaneous locomotor activity.
Values are expressed as mean ± standard error of mean (SEM, n=6).
Analyzed by one-way ANOVA followed by post hoc Dunnetts test.
***(p<0.001) vs sham control group.
+++(p<0.001) vs SNL control group

Effect of PN on transfer latency time

Compared with sham control group, the animals in SNL group showed significant (p<0.001) increment in transfer latency time. Whereas transfer latency time was significantly (p<0.001) attenuated by PN 50 and 100 mg/kg pretreated groups (Figure 5).

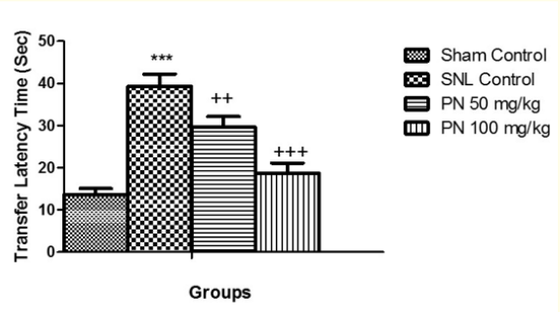

Figure 5: Effect of PN on transfer latency time.
Values are expressed as mean ± standard error of mean (SEM, n=6).
Analyzed by one-way ANOVA followed by post hoc Dunnetts test.
(p<0.01) *(p<0.001) vs sham control group.
+(p<0.05), +++(p<0.001) vs SNL control group.

Effect of PN on glutamate levels

A significant elevation (p< 0.001) of glutamate levels was observed in SNL groups as compared to the sham groups. PN pretreatment significantly (p< 0.001) prevented the increase in glutamate levels and values reached normal (Figure 6).

Figure 6: Effect of PN on glutamate levels.
Values are expressed as mean ± standard error of mean (SEM, n=6).
Analyzed by one-way ANOVA followed by post hoc Dunnetts test.
***(p<0.001) vs sham control group.
+(p<0.05), ++(p<0.01), +++(p<0.001) vs SNL control group.

Effect of PN on total calcium

Calcium levels were found to be increased in SNL control group when compared with sham control group. However, PN pre-treatment significantly (p<0.001) prevented the SNL induced increase in calcium levels when compared with SNL control group and values reached normal (Figure 7).

Figure 7: Effect of PN on calcium levels.
Values are expressed as mean ± standard error of mean (SEM, n=6).
Analyzed by one-way ANOVA followed by post hoc Dunnetts test.
(p<0.01), *(p<0.001) vs sham control group.
+++(p<0.001) vs SNL control group.

Effect of PN on lipid peroxidation

Lipid peroxidation was assessed in terms of MDA levels. A significant increase (p<0.001) in the content of MDA was observed in SNL group as compared with sham group. PN 50 and 100 mg/kg pretreated groups has significantly restored (p<0.05) the MDA levels as compared with SNL group (Figure 8).

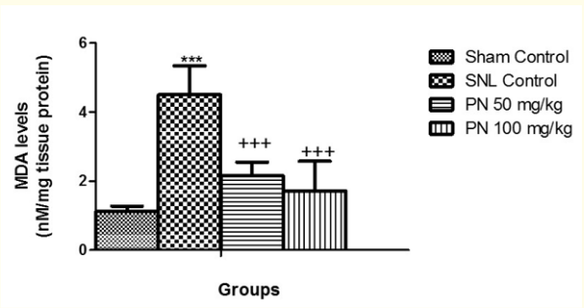

Figure 8: Effect of PN on malondialdehyde levels.
Values are expressed as mean ± standard error of mean (SEM, n=6).
Analyzed by one-way ANOVA followed by post hoc Dunnetts test.
***(p<0.001) vs sham control group.
+(p<0.05), ++(p<0.01), +++(p<0.001) vs SNL control group.

Effect of PN on reduced glutathione levels

GSH levels were found be decreased significantly ($p<0.001$) in SNL group, which was significantly ($p<0.001$) attenuated with PN 50 and 100 mg/kg pretreated groups when compared with SNL control group (Figure 9).

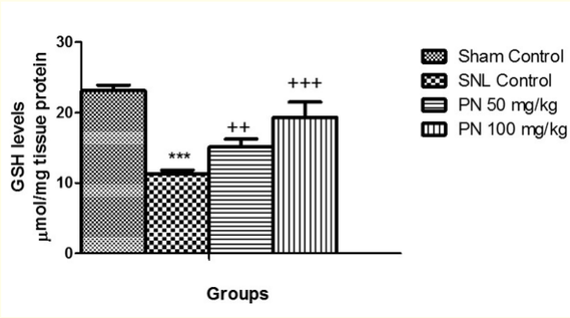

Figure 9: Effect of PN on reduced glutathione levels.
Values are expressed as mean ± standard error of mean (SEM, n=6).
Analyzed by one-way ANOVA followed by post hoc Dunnetts test.
(p<0.01), *(p<0.001) vs sham control group.
+++(p<0.001) vs SNL control group.

Effect of PN on superoxide dismutase levels

SOD levels were found to be decreased significantly ($p<0.001$) in the SNL group as compared with the sham group. PN 50 and 100 mg/kg pretreated groups significantly ($p<0.01$) prevented the SNL induced decrease in SOD levels when compared with SNL control group (Figure 10).

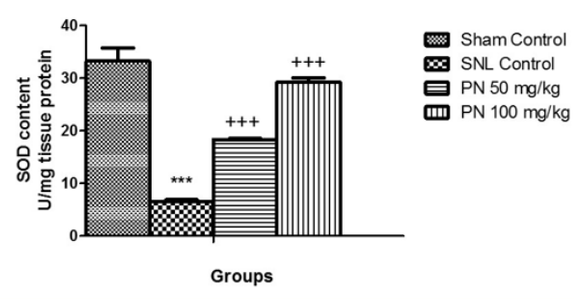

Figure 10: Effect of PN on superoxide dismutase levels.
Values are expressed as mean ± standard error of mean (SEM, n=6).
Analyzed by one-way ANOVA followed by post hoc Dunnetts test.
***(p<0.001) vs sham control group.
++(p<0.01), +++(p<0.001) vs SNL control group

Histological studies

SNL resulted in significant histological changes in the sciatic nerve. Axonal degeneration was observed in SNL group as evident by high vacuolization and decrease in number of Schwann cells. However, PN 50 and 100 mg/kg pretreated groups significantly attenuated these histological changes when compared with SNL control group (Figure 11).

Figure 11: Effect of PN on SNL induced histological changes in sciatic nerve.
(A) Sham control group. (B) SNL control group. (C) PN 50 mg/kg pre-treated group. (D) PN 100mg/kg pre-treated group.
SC- Schwann cell nuclei, NF- Nerve fibres, Ax- Axon,
DC- degenerative changes, V- Vacuolation.

Discussion

In the present study, we have evaluated the protective effect of PN in sciatic nerve ligation induced neuropathic pain in rats. Pre treatment of PN significantly decreased the behavioral and biochemical changes after SNL in rats. However, it is the first report indicating the protective role of PN against SNL induced neuropathy in rats. SNL is the widely used neuropathic pain model in rats which mimics the pain in humans [26]. Nerve injury is studied by electrophysiological and histological methods but functional evaluation is important to know the degree of injury and recovery. Foot positioning and toe spread are useful in assessing degree of injury [27]. SNL induced neuropathic pain has been assessed by measuring nociceptive threshold, allodynia, transfer latency time, foot deformity, motor coordination, exploratory behavior [28,29], which were significantly attenuated by PN treatment.

Nerve injury induced development of neuropathic pain is either sequential and/or collective function of cellular events. The

primary steps are increase of calcium ion concentration, release of proinflammatory mediators, myeloperoxidase, tumor necrosis factor-alpha from the resident macrophages. Generation of free radical and enzymatic changes eventually leading to enhancement of neuronal damage and nociceptive pain sensation [10]. SNL induced neuropathic pain has been demonstrated to produce a rise in tissue total calcium and free radicals [7,8]. Calcium ion accumulation has further been reported to trigger various secondary messengers, i.e., MPO enzyme; inflammatory cytokine (TNF-a); activation of calcium binding protein; calcium dependent kinase and phosphatase and expression of toxic proteins leading to long term potentiation, long term depression and neuronal hyper excitation [4,5,11]. Disturbance of calcium ion homeostasis has been shown to be responsible for the axonal degeneration by alteration of stability of axonal cytoskeleton proteins [30,31]. In congruent with this, our study observed increased levels of calcium in SNL induced rats, which was significantly prevented and attenuated by PN pre-treatment, respectively the calcium induced axonal degeneration and thereby alleviated the pain, which is supported with our histological findings.

An increase in calcium level triggers free radical generation and hence increases the oxidative stress [32-34]. Existing evidence has indicated involvement of oxidative stress in the pathogenesis of neuropathic pain [10]. Superoxide is produced at a relatively high rate by cells during normal metabolism / its low intra cellular level is maintained by spontaneous dismutation and/or catalytic breakdown by the enzyme superoxide dismutase (SOD). This enzyme is implicated as an essential defense against the potential toxicity of oxygen [35]. The radical scavenging activity of SOD is effective only when it is followed by actions of catalase and glutathione peroxidase as SOD generates hydrogen peroxide as a metabolite, which is more toxic than oxygen radical and requires to be scavenged by, catalase/glutathione peroxidase [36]. Considerable evidence convinces antioxidants having a vivid role in the management of neuropathic pain in rats [37-39]. In support of this, in the present study, SOD, Catalase, GSH levels were decreased and MDA levels were increased after SNL in rats which were significantly attenuated with PN pretreatment indicating the antioxidant role of PN in in alleviation of SNL induced neuropathic pain.

Conclusion

In conclusion, the present study demonstrated the protective effect of PN against SNL induced neuropathic pain as evident by improving the nociceptive threshold, attenuating the behavioral changes, and biochemical changes. The anti-allodynia, anti-hyperalgesia and antioxidant's potential may be contributed to the PN in alleviating the SNL induced hyperalgesia, allodynia, inflammation and oxidative stress. Thus, it appears to be a therapeutic alternative approach to the patients suffering from neuropathic pain.

Bibliography

1. Rahbardar GM., *et al*. "Antiinflammatory effects of ethanolic extracts of Rosmarinus officinalis L. and rosmarinic acid in a rat model of neuropathic pain". *Biomedicine and Pharmacotherapy* 86 (2017): 441-449.

2. Treede RD., *et al*. "Neuropathic pain: redefinition and a grading system for clinical and research purposes". *Neurology* 70.18 (2008): 630-1635.

3. Liang X., *et al*. "Effects of ralfinamide in models of nerve injury and chemotherapy-induced neuropathic pain". *European Journal of Pharmacology* 823 (2018): 27-34.

4. Tanimura Y., *et al*. "Neoline is the active ingredient of processed aconite root against murine peripheral neuropathic pain model, and its pharmacokinetics in rats". *Journal of Ethnopharmacology* (2019).

5. Zhang W., *et al*. "Antinociceptive and anti-inflammatory effects of cryptotanshinone through PI3K/Akt signaling pathway in a rat model of neuropathic pain". *Chemico-Biological Interactions* 305 (2019): 127-133.

6. Lai HC., *et al*. "Baicalein attenuates neuropathic pain and improves sciatic nerve function recovery in rats with partial sciatic nerve transection" *Journal of the Chinese Medical Association* 81.11 (2018): 955-963.

7. Sona WS., *et al*. "Structural hybridization of pyrrolidine based T-type calcium channel inhibitors and exploration of their analgesic effects in a neuropathic pain model". *Bioorganic and Medicinal Chemistry Letters* 29 .10 (2019): 1168-1172.

8. Sekiguchi F., *et al*. "Blockade of T-type calcium channels by 6-prenylnaringenin, a hop component, alleviates neuropathic and visceral pain in mice". *Neuropharmacology* 138 (2018): 232-244.

9. Yu G., *et al*. "Brucine alleviates neuropathic pain in mice via reducing the current of the sodium channel". *Journal of Ethnopharmacology 233 (2018): 56-63.*

10. Wang X., *et al*. "Crocetin attenuates spared nerve injury-induced neuropathic pain in mice". *Journal of Pharmacological Sciences135.4* (2017): 141-147.

11. Singh H., *et al*. "Alternative treatment strategies for neuropathic pain:role of indian medicinal plants and compounds of plant origin - A review". *Biomedicine and Pharmacotherapy* 92 (2017): 634-650.

12. Dray A. "Neuropathic pain: emerging treatments". *British Journal of Anaesthesia* 101.1 (2008): 48-58.

13. Swamy AHMV and Patil PA. "Effect of Some Clinically Used Proteolytic Enzymes on Inflammation in Rats". *Indian Journal of Pharmaceutical Sciences* 70.1 (2008): 114-117.

14. David AC and Richard JR. "Advance in Pharmacology and Chemotherapy". New York: Academic Press (1975): 266.

15. Zhuang ZY., *et al*. "ERK is sequentially activated in neurons, microglia and astrocytes by spinal nerve ligation and contributes to mechanical allodynia in this neuropathic pain mode". *Pain* 114.1-2 (2005): 149-159.

16. Etsuko NI and Yoichi K. "Pharmacological characteristics of the hind paw weight bearing difference induced by chronic constriction injury of the sciatic nerve in rats". *Life Sciences* 84.17-18 (2009): 622-626.

17. Naik AK., *et al*. "Role of oxidative stress in pathophysiology of peripheral neuropathy and modulation by N-acetyl-L-cysteine in rats." *European Journal of Pain* 10.7 (2006): 573-579.

18. Jones BJ and Roberts DJ. "The quantitative measurement of motor incoordination in naive mice using an accelerating rotarod". *The Journal of Pharmacy and Pharmacology* 20.4 (1968): 302-304.

19. Bushnell PJ. "Behavioral effects of acute xylene inhalation in rats: autoshaping, motor activity, and reversal learning". *Neurotoxicol Teratol* 10.6 (1988): 569-577.

20. Bernt E and Bergmeyer HU. "L-glutamate UV assay with glutamate dehydrogenase and NAD". *Methods of enzymatic analysis* (1974): 1704-1715.

21. Lowry OH., *et al*. "Protein measurement with folin phenol reagent". *Journal of Biological Chemistry* 193.1 (1951): 265-275.

22. Okhawa H., *et al* "Assay for lipid peroxides in animals and tissue by thiobarbituric acid reaction". *Analytical Biochemistry* 95.2 (1979): 351-358.

23. Ellman GL. "Tissue Sulfhydryl groups". *Archives of Biochemistry and Biophysics* 82.1 (1959): 70-77.

24. Misra HP and Fridovich I. "The role of superoxide anion in the autooxidation of epinephrine and a simple assay for superoxide dismutase". *Journal of Biological Chemistry* 247.10 (1972): 3170-3175.

25. Yukari S., *et al*. "Neurologic and histopathologic evaluation after high-volume intrathecal amitriptyline". *Regional Anesthesia and Pain Medicine* 29.5 (2004): 434-440.

26. Austin PJ., *et al*. "Regulatory T cells attenuate neuropathic pain following peripheral nerve injury and experimental autoimmune neuritis". *Pain* 153.9 (2012): 1916-1931.

27. Varija D., *et al*. "Prolonged constriction of sciatic nerve affecting oxidative stressors and antioxidant enzymes in rat". *Indian Journal of Medical Research* 129.5 (2009): 587-592.

28. Aswar M., *et al*. "Protective effect of hesperetin in rat model of partial sciatic nerve ligation induced painful neuropathic pain: an evidence of anti-inflammatory and anti-oxidative activity". *Pharmacology, Biochemistry and Behavior* 124 (2014): 101-107.

29. Martinez-Navarro M., *et al*. "Influence of behavioral traits in the inter-individual variability of nociceptive, emotional and cognitive manifestations of neuropathic pain". *Neuropharmacology* 148 (2019): 291-304.

30. Glass JD., *et al*. "Very early activation of m-calpain in peripheral nerve during Wallerian degeneration". *Journal of Neurological Sciences* 196.1-2 (2002): 9-20.

31. Muthuraman A and Singh N. "Neuroprotective effect of saponin rich extract of Acorus calamus L. in rat model of chronic constriction injury (CCI) of sciatic nerve-induced neuropathic pain". *Journal of Ethnopharmacology* 142.3 (2012): 723-731.

32. Siesjo BK., *et al*. "Glutamate, calcium, and free radicals as mediators of ischemic brain damage". *The Annals of thoracic surgery* 59.5 (1995): 1316-1320.

33. Carriedo SG., *et al*. "AMPA exposures induce mitochondrial Ca (2+) overload and ROS generation in spinal motor neurons in vitro". *The Journal of Neuroscience* 20.1 (2000): 240-250.

34. González A., *et al*. "Ethanol stimulates ROS generation by mitochondria through Ca2+ mobilization and increases GFAP content in rat hippocampal astrocytes". *Brain Research* 1178 (2007): 28-37.

35. Manuel R and Francisco JO. "Eight week moderate exercise training program increases plasmatic total antioxidant status in rats stressed by immobilization". *Haema* 9.1 (2006): 124-126.

36. Harman D. "The aging process major risk factor for health and disease". *Proceedings of National Academy of Sciences* 88.12 (1991): 5360-5363.

37. Low PA., *et al*. "The roles of oxidative stress and antioxidant treatment in experimental diabetic neuropathy". *Diabetes* 46.2 (1997): S38-S42.

38. Tal M. "A novel antioxidant alleviates heat hyperalgesia in rats with an experimental painful peripheral neuropathy". *Neuro report* 7.8 (1996): 1382-1384.

39. Varkonyi T., *et al*. "Current options and perspectives in the treatment of diabetic neuropathy". *Current Pharmaceutical Design* 19.27 (2013): 4981-5007.

Volume 2 Issue 7 July 2019