Life Sciences 150 (2016) 50–60

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

PARP inhibition attenuates neuroinflammation and oxidative stress in chronic constriction injury induced peripheral neuropathy

Prashanth Komirishetty [a], Aparna Areti [a], Veera Ganesh Yerra [a], Ruby PK [b], Shyam S. Sharma [b], Ranadeep Gogoi [c], Ramakrishna Sistla [d], Ashutosh Kumar [a,*]

[a] Department of Pharmacology and Toxicology, National Institute of Pharmaceutical Education and Research (NIPER)-Hyderabad, Balanagar, India
[b] Molecular Neuropharmacology Laboratory, Department of Pharmacology and Toxicology, National Institute of Pharmaceutical Education and Research (NIPER), Punjab, India
[c] Department of Biotechnology, National Institute of Pharmaceutical Education and Research (NIPER), Guwahati, India
[d] Medicinal Chemistry and Pharmacology Division, Indian Institute of Chemical Technology (IICT), Hyderabad, India

ARTICLE INFO

Article history:
Received 12 October 2015
Received in revised form 12 February 2016
Accepted 23 February 2016
Available online 24 February 2016

Keywords:
Neuroinflammation
3-Aminobenzamide
1,5-Isoquinolinediol
NF-κB
Nitrotyrosine and PAR

ABSTRACT

Aim: Peripheral nerve degeneration after nerve injury is accompanied with oxidative stress that may activate poly ADP-ribose polymerase (PARP, DNA repair enzyme). PARP overactivation amplifies the neuronal damage either due to energy crisis or through inflammatory process by facilitating nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB). Hence investigated the role of PARP inhibitors, 3-Aminobenzamide (3-AB) and 1,5-isoquinolinediol (ISO) in the attenuation of chronic constriction injury (CCI) induced peripheral neuropathy in rats.

Methods: 3-AB and ISO (at doses 30 and 3 mg/kg i.p., respectively) were tested in rats subjected to standard tests for evaluating hyperalgesia and allodynia. Sciatic functional index (SFI) was assessed by performing walking track analysis. Oxidative stress and inflammation induced biochemical alterations were estimated after 14 days in sciatic nerve and lumbar spinal cord. Molecular changes were explored by immunohistochemistry and DNA fragmentation by TUNEL assay.

Key findings: Treatment significantly improved sensorimotor responses ($p < 0.001$), SFI ($p < 0.001$) and foot posture. PARP inhibition significantly ($p < 0.01$ and $p < 0.001$) reduced the elevated levels of nitrite, inflammatory markers and also normalized the depleted $NAD_{(total)}$ levels. The protein expression of poly (ADP-ribose) (PAR), NF-κB, cyclooxygenase-2 (COX-2) and nitrotyrosine were significantly ($p < 0.01$ and $p < 0.001$) decreased in both sciatic nerve and lumbar spinal cord, evident through immunohistochemistry.

Significance: Present study outcomes fortify the pathological role of PARP overactivation in CCI induced neuropathy and PARP inhibition ameliorated oxidative stress and neuroinflammation associated with CCI induced nerve injury. Therefore, the current study suggests the PARP inhibitors can further be evaluated for designing futuristic strategies for the management of trauma induced neuropathy.

1. Introduction

Neuropathic pain, a consequence of nerve injury of peripheral nervous tissue reduces the quality of life, decreases ability to participate in daily routines and makes the person unfit for work [1]. Clinical symptoms of neuropathic pain are characterized by abnormalities in the pain sensation that are stimulus independent like shooting pain, burning, tingling and numbness. Whereas, stimulus evoked pain perceptions include thermal and mechanical hyperalgesia, tactile allodynia and sometimes may even leads to hypoalgesia [2]. Traumatic peripheral nerve injury frequently produces persistent debilitating pain states. It results in structural and functional alterations in peripheral nerve endings, afferent fibers, dorsal root ganglion (DRG) and central afferent terminals in the spinal cord resulting in sensory nociceptor sensitization, ectopic discharges, central sensitization and disinhibition [3]. Though enormous efforts have been made to find treatments for neuropathic pain, there is no therapeutic intervention which can truly be labeled as its treatment. Current pharmacotherapy includes antidepressants, calcium channel alpha-2 delta subunit blockers, selective serotonin reuptake inhibitors (SSRIs), sodium channel blockers, tramadol, capsaicin patch etc., which can only provide symptomatic relief. Also these interventions are limited by their effectiveness and adverse effects [4]. Therefore, it is necessary to search for novel molecules that target the underlying pathophysiology.

Nerve injury induce an upsurge of inflammatory mediators at the injury site which in turn activates the glial cells, responsible for amplifying the inflammatory process by synthesis and release of proinflammatory

* Corresponding author at: Department of Pharmacology and Toxicology, National Institute of Pharmaceutical Education and Research (NIPER)-Hyderabad, Balanagar, Hyderabad, Telangana 500037, India.
E-mail addresses: ashutosh.niperhyd@gov.in, ashutoshniper@gmail.com (A. Kumar).

http://dx.doi.org/10.1016/j.lfs.2016.02.085
0024-3205/

mediators. This may contribute to nitro-oxidative stress and neuroinflammation mediated neurodegeneration [5]. Elevated nitro-oxidative stress and inflammatory processes may leads to massive DNA damage. PARP after sensing the DNA damage, it gets activated and repairs the DNA by transferring ADP-ribose units to the nuclear proteins. A prominent role of PARP overactivation in the activation of inflammatory pathways and neurodegeneration has been demonstrated in various types of neuropathic pain [6–8]. Therefore, PARP inhibitors can serves as an attractive tool to counter the inflammation related nerve damage in addition to their PARP inhibitory activity.

Recently, PARP inhibitors have been reported for their potential in the treatment of other forms of neuropathies [9–11]. 3-Aminobenzamide (3-AB) is analogue of nicotinamide have long served as benchmark inhibitors of PARP widely used for investigations. It has been reported for its therapeutic efficacy in various cardiovascular diseases, diabetes and its complications and inflammatory diseases [12,13]. 1,5-Isoquinolinediol (ISO) is a derivative of isoquinolinones is more potent than classical PARP inhibitors and found to be cardioprotective and neuroprotective [12,14]. Recent studies also indicated its anti-aging and potent anti-inflammatory actions [15,16]. Evidence also suggests that these PARP inhibitors have potential to attenuate diabetes induced inflammatory stress in endothelial cells and neuronal tissue [10,17]. Objective of the current study is to assess the pathological role of PARP overactivation and therapeutic potential of 3-AB and ISO in experimental model of trauma induced neuropathic pain. We used chronic constriction injury model as it is one of the most widely used models for nerve injury induced neuropathic pain that shares many pathophysiological similarities to clinically reported neuropathy and neuropathic pain [18].

2. Materials and methods

2.1. Experimental animals

Male Sprague-Dawley rats weighing 150–200 g were procured from National Institute of Nutrition (NIN), Hyderabad, India. Animals were naive to intervention treatment and experimentations at the starting of all studies and were housed under standardized conditions in animal house with free access to food and water, maintained at constant temperature of 23 °C ± 1 °C, relative humidity 55% ± 10% and 12:12 h light and dark cycle throughout the experimentation. The animal investigations were approved by IAEC and done in accordance. All the experiments were carried out at similar time of the day and performed before CCI to obtain basal readings and at 7th and 14th day after CCI to screen the therapeutic potential of the treatment.

2.2. Drugs and chemicals

All the chemicals including 3-aminobenzamide and 1,5-isoquinolinediol were procured from Sigma, USA unless specified. NAD/NADH kit was also procured from Sigma, USA. Immunohistochemistry detection kit was supplied by PathnSitu Biotechnologies Pvt Ltd, Hyderabad, India. TdT-FragEL™ DNA fragmentation detection kit purchased from Calbiochem (EMD Millipore, USA). All other chemicals and solvents used were of analytical grade. Isoflurane was purchased from Raman and Weil Pvt. Ltd, Mumbai, India. TNF-α and IL-6 rat specific ELISA kits were obtained from eBiosciences, Inc. San Diego, CA, USA.

2.3. Induction of peripheral neuropathy by chronic constriction injury (CCI)

Rat sciatic nerve was subjected to CCI according to the Bennett and Xie method [18]. Briefly, the rats were anesthetized in an induction chamber using 4% isoflurane in presence of O_2. Left hind leg was shaved and sterilized with 70% alcohol and iodine solution. An incision was made on lateral surface of left thigh, 3–4 mm below the femur. Sciatic nerve was exposed by blunt dissection of gluteus superficialis and biceps femoris muscles. Using chromic gut 4-0, suture (Ethicon, USA) four double knot ligatures were placed, each 1 mm apart, proximal to the trifurcation of the sciatic nerve. The ligatures were made tight enough not to slide along the nerve but with minimal constriction so that epineurial blood flow was not disturbed. The wound was sutured layer by layer and animals were recovered from anesthesia on a homeothermic blanket. Then the skin was sterilized with topical iodine solution.

2.4. Drugs and dosing schedule

After habituation to the test environment and baseline measurements of pain sensitivity, rats were randomized into 5 groups each comprising 6 rats. Normal control group rats were not subjected to any surgical procedure. Sham operated group- rats were subjected to surgical procedure where the sciatic nerve was exposed but not ligated, CCI control rats with ligated sciatic nerve. Two treatment groups were subjected to CCI and treated with either 3-AB (30 mg/kg i.p.) or ISO (3 mg/kg i.p.) for 14 days. Animals were assessed for behavioral changes before CCI, 7th and 14th day after the CCI. All behavioral measures were obtained by an observer who was blinded to the group assignment to avoid bias. Tests were conducted in accordance with the guidelines of the International association for the study of pain. Thereafter, 14 days post-CCI, all the animals were euthanized using CO_2 anesthesia, and the ipsilateral sciatic nerve and lumbar spinal cord were collected. Their homogenates were used to assess the biochemical alterations and expression levels of various proteins of interest.

2.5. Functional assessment

2.5.1. Sciatic functional index (SFI, walking track analysis)

To evaluate the functional effects of sciatic nerve damage SFI was calculated according to the method of Varejao et al. [19] by measuring the hind paw foot prints of walking rats preserved on a white paper. All rats were first allowed for two or three conditioned trials to walk in the walking chamber, during which they often stop to explore the corridor. Then the hind paws of the animal were dipped in Indian ink, placed and permitted to walk on a white paper in a walking pathway that end in a darkened cage. Then several measurements were taken from the foot prints left by the rat on the white paper. They are (i) longitudinal distance from the heel to the third toe, the print length (PL); (ii) horizontal distance from the first to the fifth toe, the toe spread (TS); and (iii) horizontal distance from the second to the fourth toe, the intermediary toe spread (ITS). All the three measurements were taken from the ipsilateral (I) and contralateral (C) sides. Several foot prints were obtained from each track and average values of three foot prints were considered. From these values various factors were calculated as follows to obtain the sciatic functional index (SFI). (i) Print length factor (PLF) = (IPL − CPL) / CPL; (ii) toe spread factor (TSF) = (ITS − CTS) / CTS; (iii) intermediary toe spread factor (ITF) = (IIT − CIT) / CIT. The sciatic functional index (SFI) from these factors was calculated by using the Bain-Mackinnon-Hunter (BMH) formula; $SFI = -38.3 \times PLF + 109.5 \times TSF + 13.3 \times ITF - 8.8$. An SFI of 0 indicates normal foot and −100 indicates total impairment, which would result from a complete transection of the sciatic nerve.

2.6. Behavioral studies

2.6.1. Thermal hyperalgesia (hot and cold plate tests)

Thermal hyperalgesia of the paw to both hot (52.5 °C ± 1 °C) and cold (4 °C ± 1 °C) stimuli was performed [20,21]. This response on Eddy's hot/cold plate is considered to be resultant of both central and peripheral pain mechanisms. Animals were acclimatized prior to the experiment. The latency of the first escaping sign of paw flicking, jumping or paw licking were considered as the index of the pain threshold. The cut off time 15 s and 60 s were kept in hot and cold plate tests

respectively to avoid the paw damage. Six consecutive readings were taken at an interval of 10 min.

2.6.2. Cold chemical allodynia (acetone spray test)

Cold-allodynia of the rat hind paw was assessed using acetone spray method [22] by placing the rat on a wire mesh, spraying 100 μl of acetone onto the surface of the paw without touching the paw skin. The response of the rat was noted for 20 s and graded to a 4-point scale. 0, no response; 1, quick withdrawal or flicking of the paw; 2, prolonged withdrawal or repeated flicking; and 3, repeated flicking and licking of the paw. With a 5 min time interval, acetone was applied thrice on the hind paw and the individual scores noted in 20 s intervals were added to obtain a single score over a cumulative period of 60 s with a minimal score of 0 and maximum score of 9.

2.6.3. Mechanical dynamic allodynia (paint brush test)

This test explores the dynamic responses to mechanical stimulus. The rat was placed on the top of a wire mesh with grids and a smooth paint brush was used to rub the plantar area of hind paw from the heel to the toes. The withdrawal response is indicative of mechanical dynamic allodynia because normal rats never respond to this kind of stimulus. This is explained as the pain mediated through peripheral large myelinated Aβ-fibers [23,24]. The stimulus was applied five times with an interval of 5 s and the number of withdrawals was noted (between 0 and 5). The experiment was repeated for three times with a time interval of 5 min and total number of withdrawal responses was added to get a single cumulative score with minimum of 0 and maximum of 15.

2.6.4. Static mechanical hyperalgesia (Randall-Selitto analgesic apparatus)

Mechanical hyperalgesia was measured by using Randall-Selitto analgesic apparatus (IITC Life sciences, CA, USA) [25,26]. Pressure was applied on ipsilateral paw and time difference of 5 min was kept in between two consecutive readings. The paw withdrawal responses at the corresponding pressure in g were recorded. The cut off pressure was fixed as 150 g to avoid the paw damage.

2.6.5. Spontaneous pain assessment and assessment of foot deformation

To assess the ongoing spontaneous pain, rat was observed for 10 min in its own place. The duration of lifting, flicking and licking of the ipsilateral hind paw was noted and the cumulative duration of these responses within the 10 min duration was measured. These responses of the hind paw as a part of grooming behavior were not taken into consideration. They indicate the spontaneous ongoing pain due to peripheral nerve injury and rat adjusts its weight bearing.

Posture of the foot was observed for its deformation by placing rat on a glass surface. The foot deformation was scored as follows: 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 [27].

2.7. Biochemical estimation

All the groups of rats were euthanized after 30 min of last dose of treatment after 14th day of experimental protocol. Immediately, segments of ipsilateral sciatic nerve proximal and distal to the site of injury and L4–L6 part of spinal cord were collected and stored at −80 °C. The sciatic nerve and spinal cord homogenates were prepared using Tris-HCl (pH-7.4) buffer containing protease cocktail inhibitor (Sigma, USA) and supernatant was collected after centrifugation at 12,000 rpm and 4 °C for 15 min. These were used for further determination of malondialdehyde (MDA), nitrite, glutathione (GSH), superoxide dismutase (SOD) and nicotinamide adenine dinucleotide ($NAD_{(total)}$) levels. Total protein content of homogenates was estimated using protein assay kit (Bio-Rad, USA) using Bovine serum albumin (BSA) as a standard. Extent of lipid peroxidation was assessed by measuring 'Thiobarbituric Acid Reactive Substances' (TBARS) [28]. Absorbance was measured at 532 nm and results were expressed as μmol/mg protein. Nitrite levels were estimated at 548 nm using the Griess reagent (Sigma, USA) and were expressed as total nitrite levels as nmol/mg of protein after protein normalization [25]. Glutathione (GSH) levels were calculated using 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB/Ellman's regent) at 412 nm [29]. SOD levels were estimated using sigma kit (Sigma, USA) according to the manufacturer's instructions and expressed as units/mg of protein. Total quantity of GSH was calculated by means of a calibration curve, normalized to the protein concentration and expressed as μmol/mg.

2.7.1. Estimation of (total NADH and NAD^+) $NAD_{(total)}$ levels

The $NAD_{(total)}$ levels were measured as a mean it directly determines the PARP overactivation as evaluated by others previously [11]. The tissue supernatant was assayed for $NAD_{(total)}$ levels using sigma kit according to the manufacturer's instructions. $NAD_{(total)}$ levels were measured at 450 nm using multimode microplate reader (Spectramax M4, Molecular Devices LLC, Sunnyvale, California, USA). The total quantity of $NAD_{(total)}$ was calculated by means of a calibration curve and normalized to the protein concentration, which was estimated using a Bio-Rad protein assay kit and expressed as ng/mg of protein.

2.7.2. Estimation of inflammatory markers (TNF-α and IL-6)

For estimation of pro-inflammatory cytokines in sciatic nerve and spinal cord, homogenates were prepared using Tris-HCl (pH-7.4) buffer containing protease cocktail inhibitor (Sigma, USA) and supernatant was collected after centrifugation at 4000 rpm and 4 °C for 15 min. The supernatants were immediately used for the estimation of TNF-α and IL-6 using commercially available rat specific enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions. Cytokine levels were calculated by comparing samples to the standard curve produced from the respective kit and were expressed as pg/mg of protein after normalizing the protein content.

2.8. Histopathological evaluation

Collected tissues of distal portions of sciatic nerve and lumbar portion of the spinal cord were immediately preserved in 10% buffered formalin. These tissues were dehydrated in series of diluted alcohol, embedded in paraffin and a series of 4 μm sections were made using microtome (Leica biosystems RM2255, Wetzlar, Germany). Staining was done by using hematoxylin and eosin. Then the sections were observed under microscope (Nikon Eclipse TE2000-E, CA, USA) and analyzed for axonal degeneration and other histopathological changes.

2.9. DNA fragmentation and protein expression studies

2.9.1. TUNEL assay

Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay was used to study DNA fragmentation. Isolated sciatic nerve and spinal cord were dehydrated, embedded in paraffin and a series of 4 μm sections were made using microtome (Leica biosystems RM2255, Wetzlar, Germany). The 3′ end of fragmented DNA was labeled with fluorescein isothiocyanate (FITC) using DNA fragmentation detection kit TdT-FragEL as per manufacturer's instructions (Calbiochem, CA, USA). Sections were then mounted with mounting media containing nuclear counter stain 4′,6-diamidino-2-phenylindole (DAPI), total cells were observed under fluorescent microscope (Nikon Eclipse TE2000-E, USA). Total cell population and TUNEL-positive cells were counted using the image analysis software by (Image J 1.36; Wayne Rasband, National Institutes of Health, MD, USA). TUNEL-positive cells were expressed as percentage of total cells.

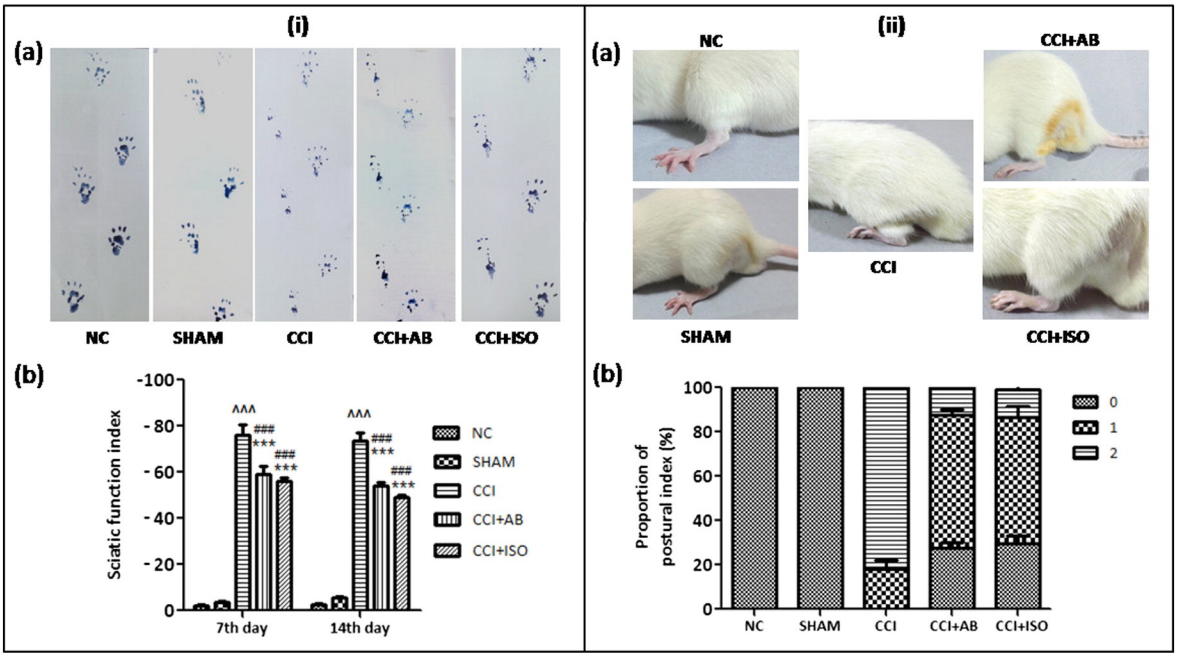

Fig. 1. Effect of 3-AB and ISO treatment on sciatic nerve function and foot posture: (i) (a) foot prints and (i) (b) SFI. (ii) (a) Pictures of ipsilateral paw postures and (ii) (b) foot deformity in terms of postural index. Results are expressed as mean ± SEM (n = 6). NC: normal control, SHAM: sham operated, CCI: CCI operated, CCI + AB and CCI + ISO: CCI rats treated with 3-AB and ISO at 30 and 3 mg/kg i.p. respectively. $^{\wedge\wedge\wedge}p < 0.001$ vs. SHAM, $^{***}p < 0.001$, vs. CCI and $^{\#\#\#}p < 0.001$ vs. SHAM on 7th and 14th day.

2.9.2. Immunohistochemistry (IHC)

The slides were warmed on a hot plate at 37 °C ± 5 °C and then deparaffinized using xylene. Then they were rehydrated with series of diluted alcohol. Later they were washed with immuno-wash buffer (pH-7.4) and incubated in blocking solution for 20 min to avoid the non-specific binding. Sections were then incubated with primary antibodies to NF-κB, COX-2 (Cell Signaling Technology, Beverly, MA, USA) in dilutions of 1:200, Nitrotyrosine and PAR (Santa Cruz Biotechnology, Texas, USA) in dilutions of 1:200 for 2 h at room temperature in a humidified chamber. Then sections were washed for 2–3 times and binding of the antibodies to their antigenic sites in sections was amplified using Poly Excel HRP/DAB Detection System (PathnSitu Biotechnologies Pvt Ltd, India). The antigen-antibody reaction sites were visualized using 3,3-diaminobenzidine (DAB) for 5 min and subsequently, sections were counterstained with Mayer's modified hematoxylin (Sigma, USA). Then dehydrated, mounted with mounting media and observed under microscope (Nikon Eclipse TE2000-E, USA) to count the stained brown cells. Total cell population and stained positive cells were counted using the image analysis software (Image J 1.36; Wayne Rasband, National Institutes of Health, MD, USA). Specific protein expressed positive cells were expressed as percentage of total cells.

2.10. Statistical analysis

The data obtained were expressed as the mean ± standard error of mean (SEM). The data from the behavioral results were statistically analyzed by two-way analysis of variance (ANOVA) and data from the biochemical results were statistically analyzed by one-way analysis of variance using the GraphPad Prism Version-5.0 software. Statistical comparisons were made with "Bonferroni's Multiple Comparison Test". Results with p values < 0.05 were considered as statistical significant.

3. Results

3.1. General behavioral observations

There were no significant changes observed in rats either subjected to CCI, sham operated group or treated groups but CCI operated rats showed general behavior like abnormal gait, posture, licking and flicking of the ipsilateral hind paw from 3rd day of the surgery. The food intake of operated rats was found to be decreased non-significantly in the initial days of surgery which might be due to the spontaneous pain and it was slowly normalized.

3.2. Effect of 3-AB and ISO treatment on functional recovery of injured sciatic nerve in CCI rats

The CCI of sciatic nerve in rats induces functional impairments which was assessed by measuring sciatic functional index using foot prints (Fig. 1(i, a)). In CCI rats, a significant ($p < 0.001$) functional impairment on 7th and 14th day was observed as compared to the sham group (CCI −76.15 ± 4.18% vs. SHAM −3.5 ± 0.76% and CCI −73.66 ± 3.26% vs. Sham −5.5 ± 2.24% on 7th and 14th day respectively). Administration of 3-AB and ISO (30 and 3 mg/kg, i.p.) for 14 days significantly ($p < 0.001$) improved the functional index (CCI + AB −54.16 ± 1.69% and CCI + ISO −49.16 ± 0.83%) (Fig. 1(i, b)).

3.3. Effect of 3-AB and ISO treatment on hyperalgesia and allodynia in CCI induced neuropathic pain

The CCI of sciatic nerve resulted in the significant ($p < 0.001$) development of thermal hyperalgesia, chemical induced cold allodynia, mechanical dynamic allodynia and mechanical hyperalgesia at day 7 and maximum effect was observed by 14th day as compared to sham group, assessed by standard methods, Eddy's hot and cold plate, acetone drop, paint brush and Randall-Selitto tests respectively. Administration

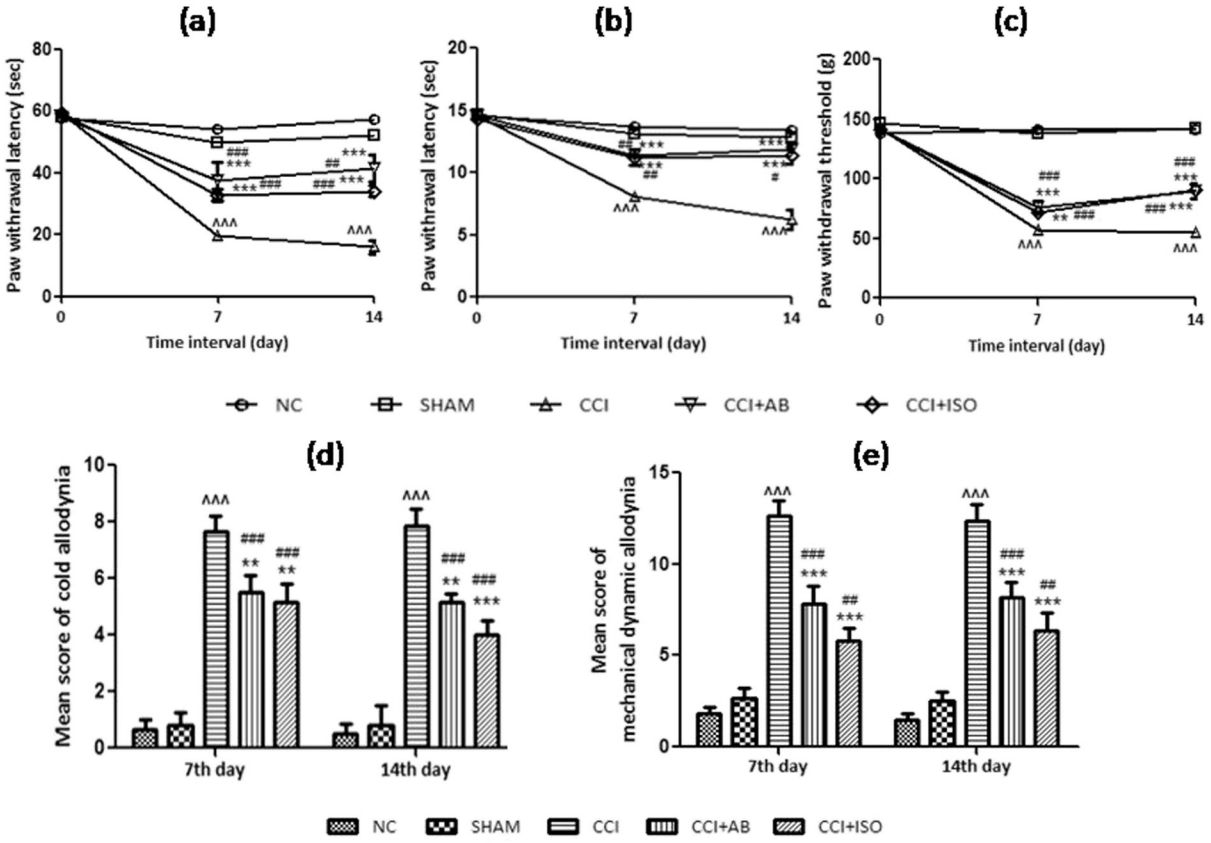

Fig. 2. Effect of 3-AB and ISO treatment on behavioral changes: (a) cold (4 °C ± 1 °C) hyperalgesia, (b) heat (52.5 °C ± 1 °C) hyperalgesia, (c) mechanical hyperalgesia, (d) cold allodynia, and (e) mechanical dynamic allodynia. Results are expressed as mean ± SEM (n = 6). NC: normal control, SHAM: sham operated, CCI: CCI operated, CCI + AB and CCI + ISO: CCI rats treated with 3-AB and ISO at 30 and 3 mg/kg i.p. respectively. $^{\wedge\wedge\wedge}p < 0.001$ vs. SHAM, $^{***}p < 0.001$ and $^{**}p < 0.01$ vs. CCI and $^{\#\#\#}p < 0.001$, $^{\#\#}p < 0.01$, and $^{\#}p < 0.05$ vs. SHAM on 7th and 14th day.

of 3-AB and ISO at doses 30 and 3 mg/kg, i.p. respectively for 14 days significantly ($p < 0.001$) increased the paw withdrawal latencies to hot and cold stimuli when compared to the CCI control animals (Fig. 2(a and b)). CCI also significantly ($p < 0.001$) increased the responses to paint brush and acetone induced cold stimuli at even 7th day and the response peaked by 14th day. Treatment for 14 days significantly ($p < 0.01$ and $p < 0.001$) reduced these CCI increased responses (Fig. 2(d and e)). Whereas the treatment also significantly increased ($p < 0.001$) the CCI elevated latencies in Randall-Selitto test (Fig. 2(c)).

3.4. Effect of 3-AB and ISO treatment on CCI induced spontaneous pain and foot deformation

In chronic constriction injury subjected rats, the cumulative lifting (39.26 ± 2.60 s) and (89.75 ± 4.50 s) along with licking duration (14.07 ± 0.98 s) and (20.58 ± 2.14 s) was observed during the time interval of 10 min on 7th and 14th day respectively. These durations were significantly ($p < 0.001$) higher as compared to lifting duration on 7th (7.84 ± 1.11 s) and 14th day (6.52 ± 1.14 s) along with licking duration on 7th (1.33 ± 0.24 s) and 14th day (2.08 ± 0.32 s) in sham control group. Treatment with 3-AB and ISO at doses 30 and 3 mg/kg, i.p. respectively, decreased the duration of cumulative lifting (31.15 ± 2.57 s and 29.21 ± 3.46 s) on 7th day and (26.49 ± 2.44 s and 28.83 ± 2.97 s) on 14th day along with licking duration (6.32 ± 0.78 s and 6.43 ± 0.84 s) on 7th day and (7.81 ± 0.66 s and 7.40 ± 1.02 s) on 14th day was observed suggesting the significant ($p < 0.001$) attenuation of chronic constriction injury induced spontaneous pain.

In CCI subjected rats, significant postural defects in terms of foot deformation was observed as compared to near normal postural index of sham group. Administration of 3-AB and ISO at doses 30 and 3 mg/kg, i.p., respectively corrected the CCI-induced foot deformity (Fig. 1(ii, a and ii, b)).

3.5. Effect of 3-AB and ISO treatment on CCI induced nitro-oxidative stress markers

The chronic constriction injury of sciatic nerve resulted in elevated levels of nitro-oxidative stress marker (MDA and nitrite levels) and decreased the GSH levels significantly ($p < 0.001$). Treatment with 3-AB and ISO (30 and 3 mg/kg, i.p.) significantly decreased the nitro-oxidative stress induced lipid peroxidation and nitrite formation in sciatic nerve ($p < 0.01$ and $p < 0.001$ respectively) and spinal cord ($p < 0.01$ and $p < 0.001$ respectively) (Fig. 3(a and b)). GSH levels were non-significantly restored in both the tissues (Fig. 3(c)). Significant ($p < 0.01$) increase was observed in the levels of antioxidant enzyme SOD in sciatic nerve and spinal cord of CCI animals when compared to sham animals. However, SOD levels remained unaltered by the treatment (Fig. 3(d)).

3.6. Effect of 3-AB and ISO treatment on $NAD_{(total)}$ levels

PARP overactivation causes excessive depletion of total NAD levels which coincides with the significant ($p < 0.001$) reduced levels in CCI group as compared to the sham group. Treatment with 3-AB and ISO (30 and 3 mg/kg, i.p.) significantly ($p < 0.001$) restored the NAD levels in both tissues (Fig. 3(e)).

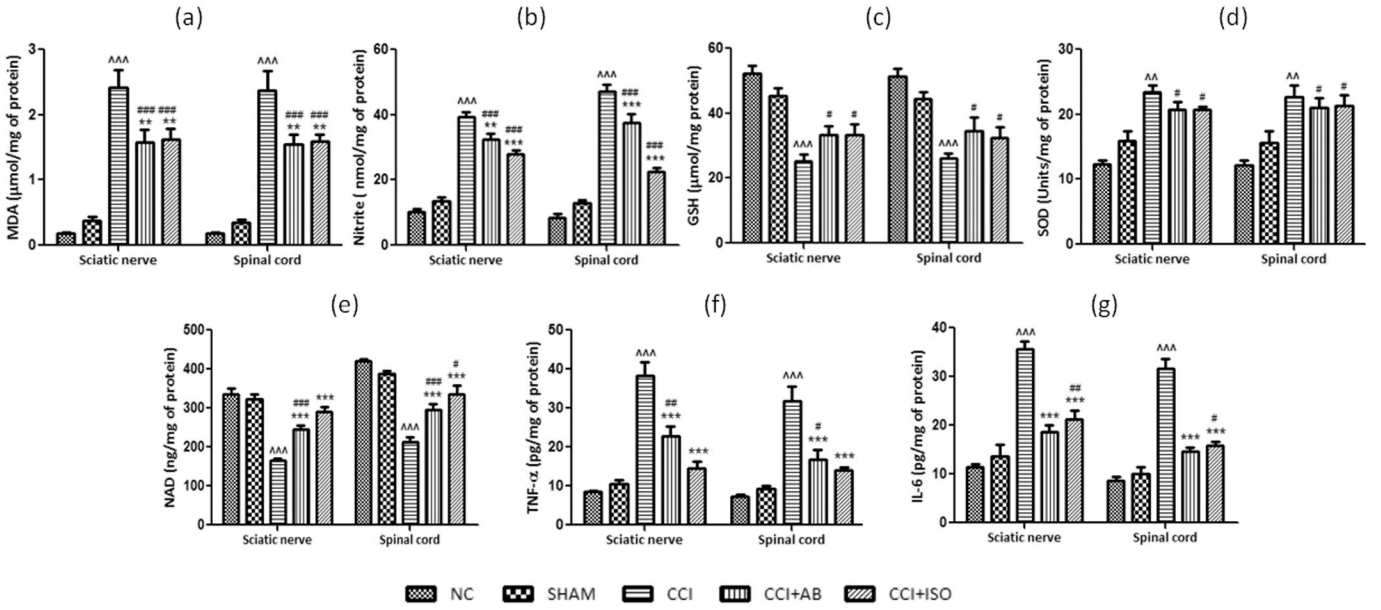

Fig. 3. Effect of 14 days treatment with 3-AB and ISO on various biochemical characteristics of CCI induced neuropathy: (a) MDA, (b) nitrite, (c) GSH, (d) SOD, (e) $NAD_{(total)}$, (f) TNF-α and (g) IL-6 levels. Results are expressed as mean ± SEM (n = 3). NC: normal control, SHAM: sham operated, CCI: CCI operated, CCI + AB and CCI + ISO: CCI rats treated with 3-AB and ISO at 30 and 3 mg/kg i.p. respectively. $^{\wedge\wedge\wedge}p < 0.001$ vs. SHAM, $^{***}p < 0.001$ $^{**}p < 0.01$, and $^{*}p < 0.05$ vs. CCI and $^{\#\#\#}p < 0.001$, $^{\#\#}p < 0.01$, and $^{\#}p < 0.05$ vs. SHAM.

3.7. Effect of 3-AB and ISO treatment on the levels of inflammatory markers in CCI induced neuropathic pain

The biological markers of inflammation, we estimated TNF-α and IL-6 levels in sciatic nerve and spinal cord tissues. The chronic constriction injury of sciatic nerve significantly ($p < 0.001$) increased the TNF-α and IL-6 levels in both the tissues. A significant ($p < 0.001$) reduction was observed with 3-AB and ISO (30 and 3 mg/kg, i.p.) treatment in sciatic nerve and spinal cord (Fig. 3(f and g)).

3.8. Effect of 3-AB and ISO treatment on CCI induced histopathological changes

The nerve sections of sham operated animals were found to be normal. Significant histopathological changes were observed in the longitudinal sections of the sciatic nerve in CCI induced animals. In longitudinal sections of nerve, nerve derangement, axon degeneration, endoneurial edema and massive demyelination were observed. It was also evident that there is increase infiltration of immune cells. Animals treated with 3-AB and ISO (30 and 3 mg/kg, i.p.) for 14 days had not exhibited the complete attenuation of the CCI induced changes but restored the nerve fiber arrangement and decreased the endoneurial swelling. Whereas the transverse sections of dorsal horns of lumbar spinal cord showed axon degeneration, swelling and immune cell infiltration. Treatment with PARP inhibitors reversed these CCI induced changes and restored the normal histology of spinal cord. Images were taken under microscope (Nikon Eclipse TE2000-E, USA) (Fig. 4).

3.9. TUNEL assay

To assess the DNA fragmentation after the nerve transection TUNEL reaction was performed where the nerve and spinal cord of CCI animals

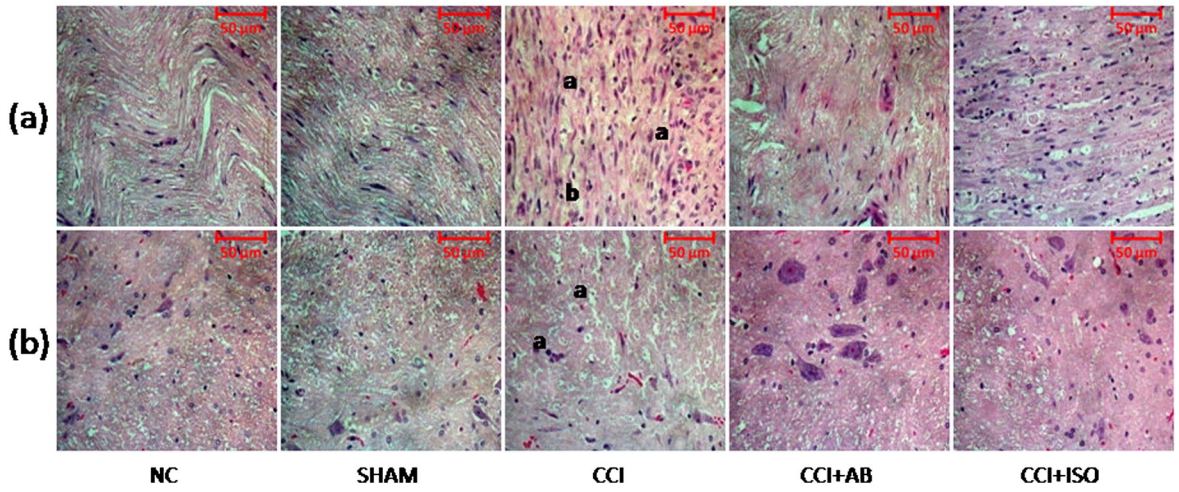

Fig. 4. Effect of 3-AB and ISO treatment on CCI induced histopathological changes: Pictorial representations of hematoxylin and eosin stained (a) sciatic nerve longitudinal sections and (b) spinal cord transverse sections. NC: normal control, SHAM: sham operated, CCI: CCI operated, CCI + AB and CCI + ISO: CCI rats treated with 3-AB and ISO at 30 and 3 mg/kg i.p. respectively. 'a' indicates nerve derangement, and 'b' indicates axonal swelling and massive demyelination. The photographs were taken at ×400. Micron bar shows a length of 50 µm.

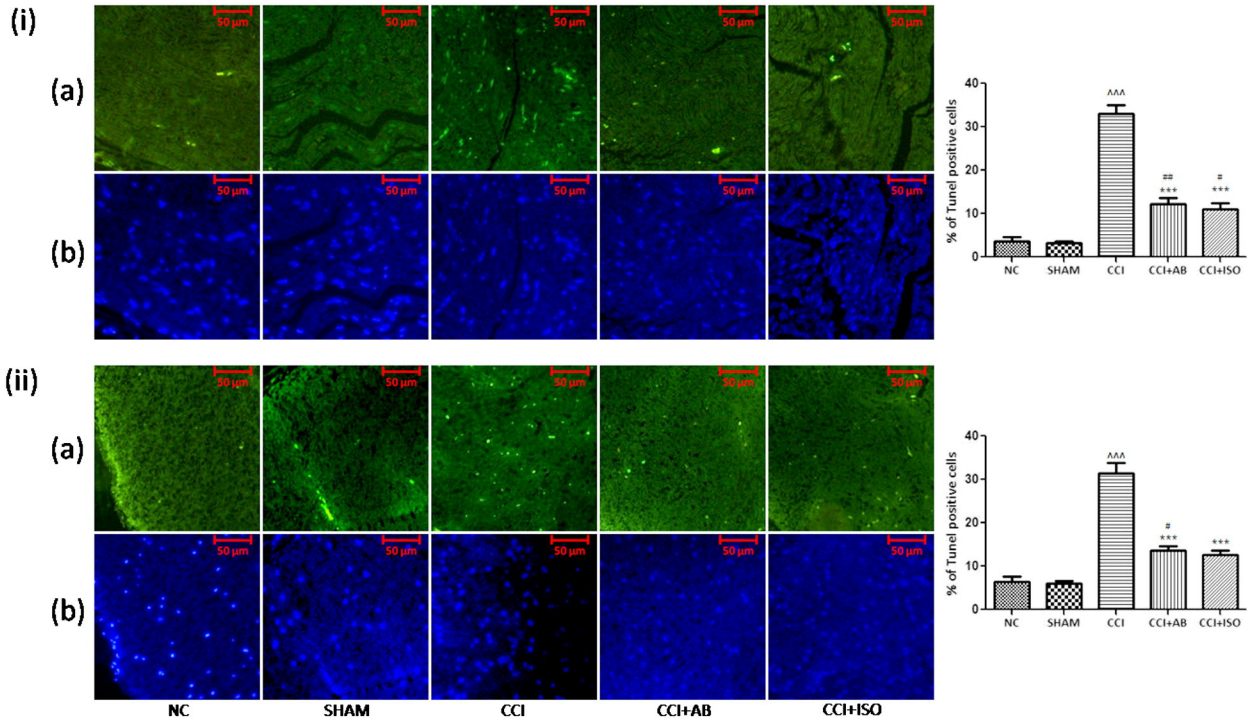

Fig. 5. Effect of 3-AB and ISO treatment on PARP overactivation induced DNA fragmentation: Pictorial representations of (i) sciatic nerve longitudinal sections and (ii) spinal cord transverse sections in which upper panel represents FITC images (a) showing TUNEL positive cells and lower panel represents DAPI images (b). Bar graphs represent the average percentage of positive cells among the total number of nuclei. Results are expressed as mean ± SEM (n = 6). NC: normal control, SHAM: sham operated, CCI: CCI operated, CCI + AB and CCI + ISO: CCI rats treated with 3-AB and ISO at 30 and 3 mg/kg i.p. respectively. ^^^$p < 0.001$ vs. SHAM, ***$p < 0.001$ vs. CCI and ##$p < 0.01$ and #$p < 0.05$ vs. SHAM. The photographs were taken at ×400. Micron bar shows a length of 50 μm.

exhibited significantly ($p < 0.001$) higher percentage of positively stained nerve resident cells, an indicative of DNA damage as compared to the nerve microsections of sham animals. TUNEL positive cells were observed in spinal cord and nerve. 3-AB and ISO (30 and 3 mg/kg, i.p.) treatment significantly ($p < 0.001$) reduced the TUNEL positive cells in both the tissue sections (Fig. 5).

3.10. Effect of 3-AB and ISO treatment on PAR, NF-κB, COX-2 and nitrotyrosine immunohistochemistry

PARP overactivation and its related inflammatory mediators in traumatic conditions aggravate the neurodegeneration process. Hence we investigated the PAR levels as an indicator of PARP overactivation, inflammatory mediator NF-κB, inflammation inducible enzyme COX-2 and nitrotyrosine as an indicator of nitro-oxidative stress in the nerve and spinal cord sections of CCI animals were assessed the effect of PARP inhibitors on the levels of these mediators. The chronic constriction injury of sciatic nerve significantly ($p < 0.001$) increased the levels of PAR and treatment with PARP inhibitors significantly ($p < 0.001$) attenuated these levels in both the tissue sections. The levels of NF-κB and COX-2 positive cells were also significantly ($p < 0.001$) elevated in the CCI animals. These were significantly ($p < 0.001$) decreased with the 3-AB and ISO (30 and 3 mg/kg, i.p.) treatment for 14 days as compared to sham group in both the tissues suggesting the abrogation of inflammatory cascades following the nerve injury. Nitrotyrosine positive cells were significantly ($p < 0.001$) increased in the sciatic nerve and spinal cord sections of CCI group when compared to sham group and treatment with PARP inhibitors significantly attenuated these levels in both the tissues (Figs. 6 and 7).

4. Discussion

The current study unravels the protective effect of two PARP inhibitors, 3-AB and ISO in the experimental nerve injury induced neuropathy. CCI of sciatic nerve produced consistent hyperalgesia and allodynia which resembles the painful conditions in patients suffering from trauma induced neuropathic pain [18]. CCI of sciatic nerve results in degeneration of axons distal and proximal to the ligation causing loss of >80% myelinated fibers and >60% of unmyelinated fibers [30]. Moreover, evidence indicates that CCI also affects the structure and function of neurons residing in L4 to L6 DRG and spinal cord entry zone, alters the excitability properties and contributes to the production of central mechanisms of hyperalgesia [31]. In the present study, CCI of sciatic nerve caused significant loss of ipsilateral hind limb function, evident through sciatic functional index based on foot print analysis. It also significantly reduced the thermal, mechanical and cold thresholds of ipsilateral hind limb assessed on 14th day suggesting altered sensorimotor parameters like hyperalgesia and allodynia. Furthermore, spontaneous pain was also examined in terms of paw flicking and licking along with foot deformity which was found to be pronounced on 14th day after surgery, which is in line with earlier published reports [32,33].

Administration of 3-AB and ISO (30 and 3 mg/kg, i.p.) for 14 days significantly recovered the sciatic function as assessed from the walking track analysis. Loss of hind limb function after the sciatic nerve constriction is due to the axon degeneration, demyelination and disturbed sensory motor inputs [34]. Improved sciatic functional index with the treatment indicate potential of PARP inhibition against the nerve injury induced neuropathy. PARP overactivation contributes to functional deficits and neurodegeneration in various types of peripheral neuropathies and neurodegenerative disorders [10,11,26]. In this study treatment with 3-AB and ISO also attenuated the CCI-induced behavioral changes including thermal hyperalgesia; cold allodynia; dynamic mechanical allodynia; mechanical hyperalgesia; spontaneous pain and postural defect in terms of foot deformity. Neuroprotective effect of these PARP inhibitors has been reported to reverse the established functional and behavioral alterations in experimental models of diabetic neuropathy [17,35]. Recent findings also suggest that PARP inhibitors like ABT-888 are capable of alleviating allodynia in chemotherapy induced

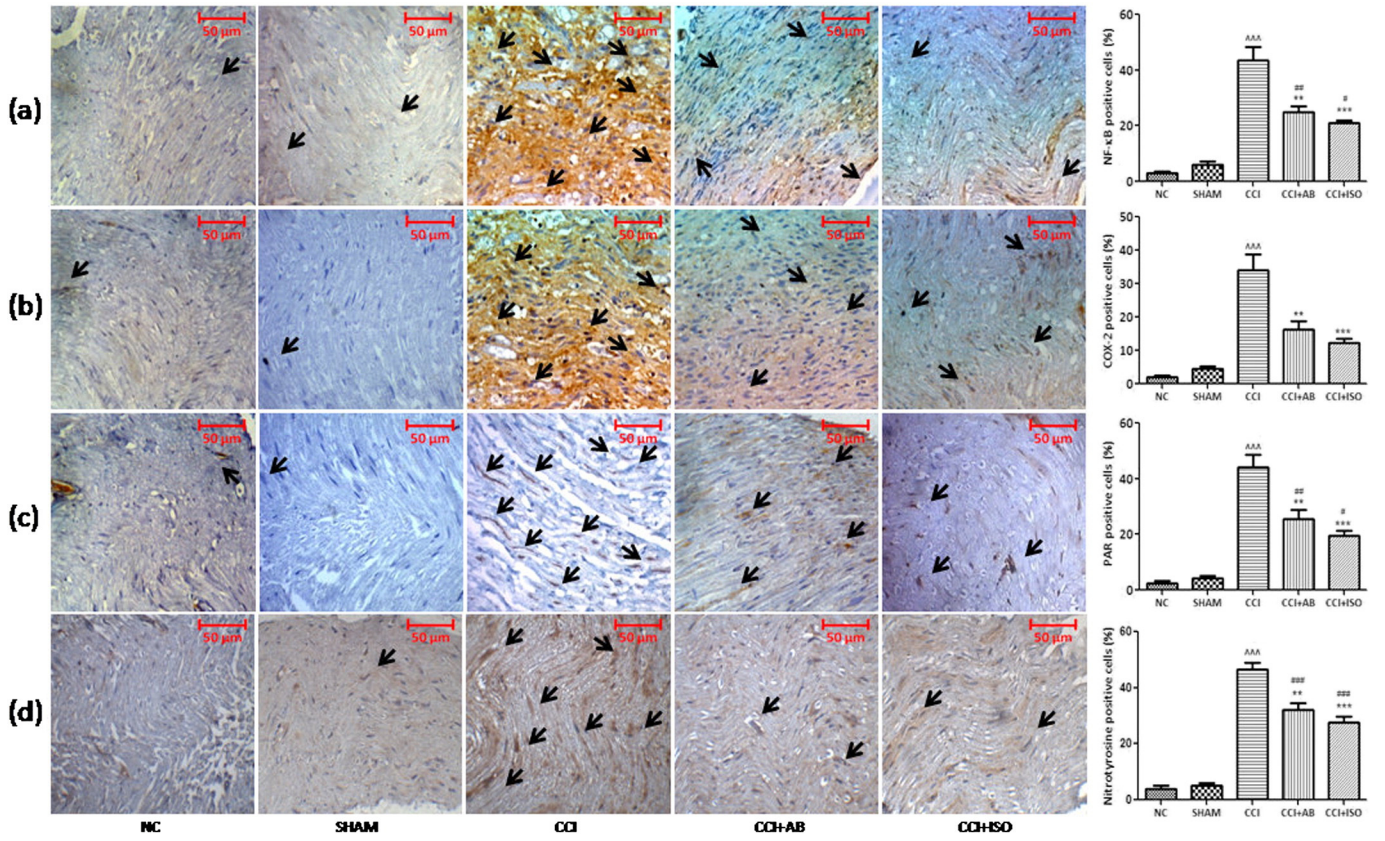

Fig. 6. Effect of 3-AB and ISO treatment on NF-κB, COX-2, PAR and nitrotyrosine immunohistochemistry in longitudinal sections of sciatic nerves: Pictorial representations of expression of NF-κB (a), COX-2 (b), PAR (c) and nitrotyrosine (d). Arrows indicate positive stained cells. Bar graphs represent the average percentage of positive cells among the total number of nuclei. Results are expressed as mean ± SEM (n = 6). NC: normal control, SHAM: sham operated, CCI: CCI operated, CCI + AB and CCI + ISO: CCI rats treated with 3-AB and ISO at 30 and 3 mg/kg i.p. respectively. $^{\wedge\wedge\wedge}p < 0.001$ vs. SHAM, $^{***}p < 0.001$ and $^{**}p < 0.01$ vs. CCI and $^{\#\#\#}p < 0.001$, $^{\#\#}p < 0.01$, and $^{\#}p < 0.05$ vs. SHAM. The photographs were taken at ×400. Micron bar shows a length of 50 μm.

neuropathy models [8,9]. We for the first time report the ameliorative potential of PARP inhibitors, 3-AB and ISO in CCI induced neuropathy.

Though lot of research has been accomplished in the field of the neuropathic pain, still the exact underpinning pathomechanism is unclear and there is no approved drug which can mitigate the pathomechanism behind neuropathic pain [36]. As neuropathic pain itself is a challenging clinical presentation to manage, investigation into basic mechanism underlying neuropathic pain and development of new therapeutic strategies are still warranted [37]. It has been well revealed that following the peripheral nerve injury the amplified neuroinflammatory cascade of events with structural and functional alterations sweep over the whole peripheral nervous system including peripheral nerve endings, DRG and also the spinal cord. This may lead to events like axon degeneration, demyelination, infiltration of immune cells, cell loss leading to central sensitization and manifests as hyperalgesia and allodynia [38]. Histological studies in sciatic nerve and lumbar spinal cord revealed that changes seen in sciatic nerve and lumbar spinal cord are in accordance with the existing reports and PARP inhibition partially preserved the neuronal structure in these tissues. These observations like improved sciatic function and histology may be at least in part due to PARP inhibition [39,40].

Release of free radicals and associated oxidant induced injury in nerve and spinal cord after peripheral nerve injury has been well reported in literature [41,42]. Specifically, the elevated superoxide and nitric oxide may combine to form peroxynitrite that can eventually lead to massive DNA damage resulting in the over activation of poly (ADP-ribose) polymerase (PARP) enzyme [41,43]. Evidences also suggest the PARP activation cannot only result from oxidative damage but itself contribute to free radical and oxidant generation [17]. The over activated PARP ignites the neuroinflammatory cascade that cause stupendous release of various inflammatory mediators like NF-κB, IL-1β, IL-6, COX-2, iNOS, and TNF-α. It also augment PARP suicide mechanism, which consumes high amount of $NAD_{(total)}$ for the synthesis of poly (ADP-ribose) (PAR) units resulting in bioenergetic failure due to ATP depletion and this whole can culminate into nerve damage [44,45]. PARP induced expression of NF-κB, TNF-α, and IL-6 causes an imbalance between the excitatory currents and inhibitory feedback mechanisms. These may be responsible for outcomes like hyperalgesia and allodynia [46,47]. Nonetheless, the role of these inflammatory cytokines has been well documented in peripheral as well as central sensitization in neuropathic pain [48]. Previous scientific reports also strongly suggest the involvement of neuroinflammatory cascades in peripheral nerve and spinal cord after the nerve injury is critical in the development of neuropathic pain [49]. All together these findings driven us to hypothesize the oxidative DNA damage induced PARP overactivation and resultant progression of inflammatory cascades after the CCI play a critical role in the development of induced neuropathic pain.

In the present study, we also evaluated the neuroprotective role of PARP inhibition on various biochemical alterations assessed in the sciatic nerve and lumbar spinal cord. CCI produced significant raise in the levels of nitrites and MDA, an indicative of nitro-oxidative stress in both the tissues. Treatment with 3-AB and ISO reduced the nitro-oxidative stress markers significantly, suggesting the abrogation of nitro-oxidative stress with PARP inhibition. The recent findings also suggest the nitro-oxidative stress and PARP overactivation in other neuropathies is bidirectional rather than unidirectional [17]. Therefore, the

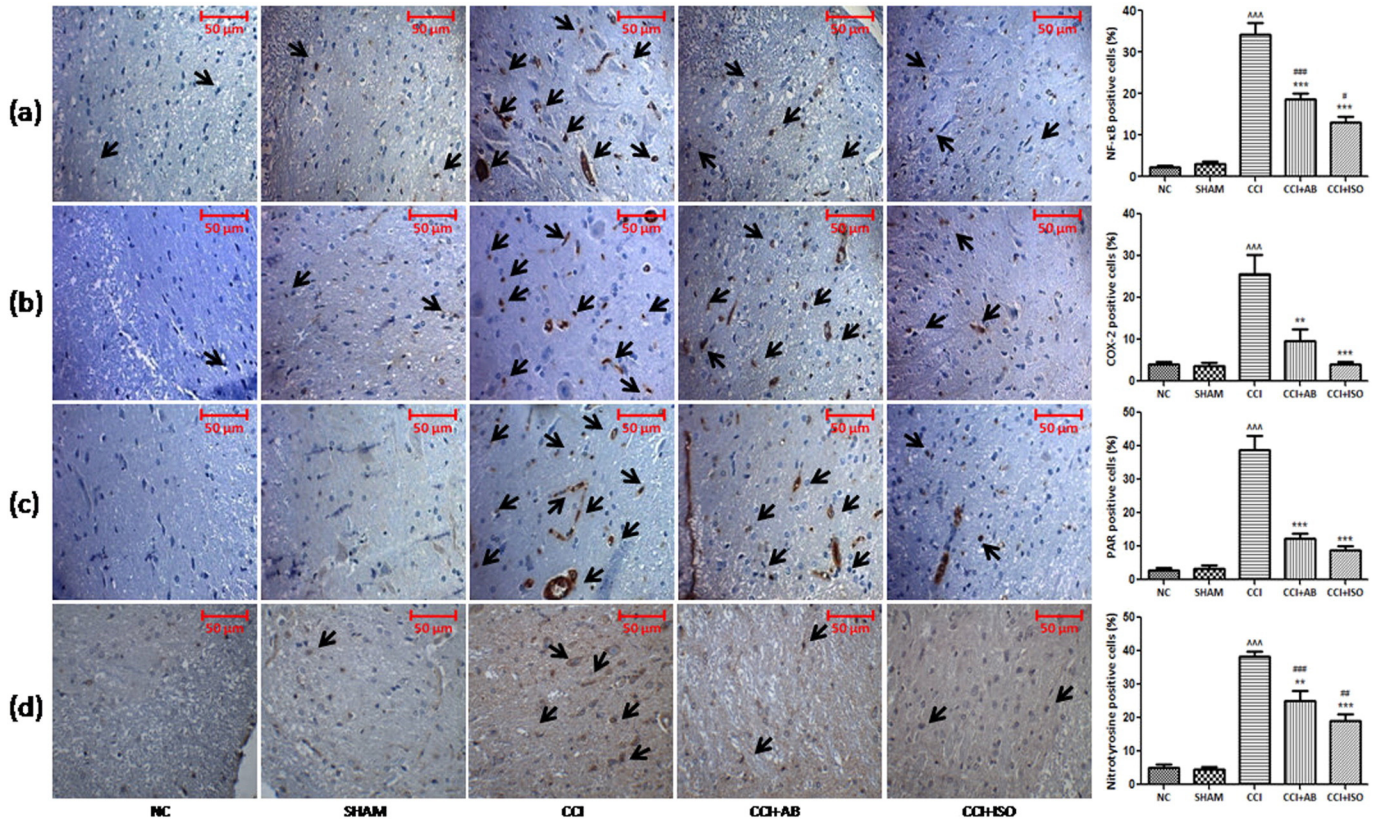

Fig. 7. Effect of 3-AB and ISO treatment on NF-κB, COX-2, PAR and nitrotyrosine immunohistochemistry in transverse sections of spinal cords: Pictorial representations of expression of NF-κB (a), COX-2 (b), PAR (c) and nitrotyrosine (d). Arrows indicate positive stained cells. Bar graphs represent the average percentage of positive cells among the total number of nuclei. Results are expressed as mean ± SEM (n = 6). NC: normal control, SHAM: sham operated, CCI: CCI operated, CCI + AB and CCI + ISO: CCI rats treated with 3-AB and ISO at 30 and 3 mg/kg i.p. respectively. $^{\wedge\wedge\wedge}p < 0.001$ vs. SHAM, $^{***}p < 0.001$ and $^{**}p < 0.01$ vs. CCI and $^{\#\#\#}p < 0.001$, $^{\#\#}p < 0.01$, and $^{\#}p < 0.05$ vs. SHAM. The photographs were taken at 400×. Micron bar shows a length of 50 μm.

treatment abrogative effect on increased levels of nitro-oxidative stress markers, restoration of GSH and expression of nitrotyrosine in CCI rats can be possibly due to PARP inhibition. CCI also resulted in up-regulation of the proinflammatory cytokines (TNF-α and IL-6) in sciatic nerve and spinal cord, which plays a critical role in the development of hyperalgesia and allodynia [47,50]. Treatment with 3-AB and ISO significantly reduced the levels of TNF-α and IL-6 which hints to anti-inflammatory action due to PARP inhibition in CCI induced neuropathy. NF-κB is one of the transcription factor related to generation of various inflammatory proteins and enzymes. PARP is essential for the activity of transcription factors such as NF-κB, hence inhibition of PARP leads to decline in NF-κB activity which in turn decreases the expression of inflammation related proteins which is in line with available reports [33,51]. Apart from these biochemical alterations, we also checked DNA fragmentation, in sciatic nerve and lumbar spinal cord microsections. CCI rats exhibited significant number of DNA fragmented nuclei in nerve resident cells that can be correlated with the nitro-oxidative stress and inflammatory damage [25,43,52,53]. However, 3-AB and ISO treatment significantly reduced the CCI induced DNA fragmentation in both the tissues suggesting the corrective effect of PARP inhibition on cell loss/death. Further, to assess the existence of PARP suicide mechanism in CCI induced neuropathy we studied the levels of $NAD_{(total)}$ and PAR immunoreactivity in sciatic nerve and lumbar spinal cord sections. We found significant reduction in $NAD_{(total)}$ levels and increased PAR expression in CCI rats and administration of PARP inhibitors significantly restored the $NAD_{(total)}$ levels as well as reduced the PAR levels in both the tissues suggesting the prevention of axonal degeneration due to the bioenergetic failure. Restoration of $NAD_{(total)}$ indicates the energy homeostasis as it prevents the excess ATP depletion which results from PARP overactivation [54]. NF-κB and COX-2 are the well-demonstrated pronociceptive molecules that are expressed in sciatic nerve and dorsal horns of spinal cord [32,33,55]. They initiate the inflammation process and involves in its resolution. For this reason, expression levels of NF-κB and COX-2 were studied in both the tissues. 3-AB and ISO treatment significantly reduced the CCI increased expression levels of NF-κB and COX-2, evident from the immunohistochemistry in sciatic nerve and spinal cord. Therefore, based on these study outcomes we can speculate that neuroprotective effect of PARP inhibition in CCI induced neuropathy is due to alleviation of nitro-oxidative stress induced DNA damage. PARP inhibitors mitigated neuroinflammation and nitro-oxidative stress induced functional loss and structural changes and showed protection against PARP-overactivation induced neuronal damage in experimental model of trauma induced neuropathy.

5. Conclusions

In summary, the current study throws light on the role of PARP overactivation and related oxidative stress and neuroinflammation in the experimental model of CCI induced neuropathy. It has been well correlated with the elevated nitro-oxidative stress markers, depleted $NAD_{(total)}$ levels, increased levels of inflammatory markers like IL-6 and TNF-α and increased expression of inflammatory mediators like NF-κB, COX-2 and PARP overactivation. PARP inhibition with 3-AB and ISO improved the functional, behavioral and biochemical deficits linked with CCI induced neuropathy and reduced the expression of NF-κB, COX-2, PAR and nitrotyrosine in sciatic nerve and spinal cord micro sections. Hence, based on the data in hand and with the support of literature, PARP inhibition by 3-AB and ISO showed beneficial effects

against peripheral nerve injury induced neuropathy, which may be attributed to their multiple effects via; antioxidant, anti-inflammatory and neuroprotective properties.

Disclosure

This study was supported by the National Institute of Pharmaceutical Education and Research, Hyderabad and Department of Biotechnology Govt of India, via grant BT/527/NE/TBP/2013.

Conflict of interest

The authors also declare no conflicts of interest.

Acknowledgement

Authors would like to acknowledge the financial support from Department of Pharmaceuticals, Ministry of Chemical and Fertilizers and NIPER Hyderabad for their support. Authors would also like to thank Department of Biotechnology Govt of India, for their financial support via grant BT/527/NE/TBP/2013, to carry out the current study.

References

[1] R. Baron, A. Binder, G. Wasner, Neuropathic pain: diagnosis, pathophysiological mechanisms, and treatment, Lancet Neurol. 9 (8) (2010) 807–819.
[2] F.T. Nickel, F. Seifert, S. Lanz, C. Maihofner, Mechanisms of neuropathic pain, Eur. Neuropsychopharmacol. 22 (2) (2012) 81–91.
[3] G. Moalem, D.J. Tracey, Immune and inflammatory mechanisms in neuropathic pain, Brain Res. Rev. 51 (2) (2006) 240–264.
[4] N.B. Finnerup, SrH Sindrup, T.S. Jensen, The evidence for pharmacological treatment of neuropathic pain, Pain 150 (3) (2010) 573–581.
[5] J. Mika, M. Zychowska, K. Popiolek-Barczyk, E. Rojewska, B. Przewlocka, Importance of glial activation in neuropathic pain, Eur. J. Pharmacol. 716 (1) (2013) 106–119.
[6] V.R. Drel, S. Lupachyk, H. Shevalye, I. Vareniuk, W. Xu, J. Zhang, N.A. Delamere, M. Shahidullah, B. Slusher, I.G. Obrosova, New therapeutic and biomarker discovery for peripheral diabetic neuropathy: PARP inhibitor, nitrotyrosine, and tumor necrosis factor-α, Endocrinology 151 (6) (2010) 2547–2555.
[7] R.K. Sodhi, N. Singh, A.S. Jaggi, Poly (ADP-ribose) polymerase-1 (PARP-1) and its therapeutic implications, Vasc. Pharmacol. 53 (3) (2010) 77–87.
[8] L.E. Ta, J.D. Schmelzer, A.J. Bieber, C.L. Loprinzi, G.C. Sieck, J.D. Brederson, P.A. Low, A.J. Windebank, A novel and selective poly (ADP-ribose) polymerase inhibitor ameliorates chemotherapy-induced painful neuropathy, PLoS One 8 (1) (2013) e54161.
[9] J.D. Brederson, S.K. Joshi, K.E. Browman, J. Mikusa, C. Zhong, D. Gauvin, X. Liu, Y. Shi, T.D. Penning, A.R. Shoemaker, PARP inhibitors attenuate chemotherapy-induced painful neuropathy, J. Peripher. Nerv. Syst. 17 (3) (2012) 324–330.
[10] F. Li, V.R. Drel, C. Szabo, M.J. Stevens, I.G. Obrosova, Low-dose poly (ADP-ribose) polymerase inhibitor-containing combination therapies reverse early peripheral diabetic neuropathy, Diabetes 54 (5) (2005) 1514–1522.
[11] S.S. Sharma, A. Kumar, R.K. Kaundal, Protective effects of 4-amino1, 8-napthalimide, a poly (ADP-ribose) polymerase inhibitor in experimental diabetic neuropathy, Life Sci. 82 (11) (2008) 570–576.
[12] J.C. Docherty, B. Kuzio, J.A. Silvester, J. Bowes, C. Thiemermann, An inhibitor of poly (ADP-ribose) synthetase activity reduces contractile dysfunction and preserves high energy phosphate levels during reperfusion of the ischaemic rat heart, Br. J. Pharmacol. 127 (6) (1999) 1518–1524.
[13] L. Virag, C. Szaba, The therapeutic potential of poly (ADP-ribose) polymerase inhibitors, Pharmacol. Rev. 54 (3) (2002) 375–429.
[14] A. Jangra, A.K. Datusalia, S.S. Sharma, Reversal of neurobehavioral and neurochemical alterations in STZ-induced diabetic rats by FeTMPyP, a peroxynitrite decomposition catalyst and 1, 5-isoquinolinediol a poly (ADP-ribose) polymerase inhibitor, Neurol. Res. 36 (7) (2014) 619–626.
[15] M.S. Park, J.-S. Choi, W. Lee, Y.J. Yang, J. Kim, G.-J. Lee, S.S. Kim, S.H. Park, S.C. Kim, J.W. Choi, Pharmacogenomic analysis indicates potential of 1, 5-isoquinolinediol as a universal anti-aging agent for different tissues, Oncotarget (2015).
[16] R. Olszanecki, A. Gebska, J. Jawien, A. Jakubowski, R. Korbut, Inhibition of NOS-2 induction in LPS-stimulated J774.2 cells, J. Physiol. Pharmacol. 57 (1) (2006) 109–117.
[17] I.G. Obrosova, V.R. Drel, P. Pacher, O. Ilnytska, Z.Q. Wang, M.J. Stevens, M.A. Yorek, Oxidative–nitrosative stress and poly (ADP-ribose) polymerase (PARP) activation in experimental diabetic neuropathy the relation is revisited, Diabetes 54 (12) (2005) 3435–3441.
[18] G.J. Bennett, Y.K. Xie, A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man, Pain 33 (1) (1988) 87–107.
[19] A.S.P. Varejao, M.F. Meek, A.J.A. Ferreira, J.A.B. Patralcio, A.M.S. Cabrita, Functional evaluation of peripheral nerve regeneration in the rat: walking track analysis, J. Neurosci. Methods 108 (1) (2001) 1–9.
[20] L. Bardin, N. Malfetes, A. Newman-Tancredi, R. Depoortere, Chronic restraint stress induces mechanical and cold allodynia, and enhances inflammatory pain in rat: relevance to human stress-associated painful pathologies, Behav. Brain Res. 205 (2) (2009) 360–366.
[21] C. Huang, Z.-P. Hu, H. Long, Y.-S. Shi, J.-S. Han, Y. Wan, Attenuation of mechanical but not thermal hyperalgesia by electroacupuncture with the involvement of opioids in rat model of chronic inflammatory pain, Brain Res. Bull. 63 (2) (2004) 99–103.
[22] S.J.L. Flatters, G.J. Bennett, Ethosuximide reverses paclitaxel-and vincristine-induced painful peripheral neuropathy, Pain 109 (1) (2004) 150–161.
[23] R.H. Gracely, S.A. Lynch, G.J. Bennett, Painful neuropathy: altered central processing maintained dynamically by peripheral input, Pain 51 (2) (1992) 175–194.
[24] J.L. Ochoa, D. Yarnitsky, Mechanical hyperalgesias in neuropathic pain patients: dynamic and static subtypes, Ann. Neurol. 33 (5) (1993) 465–472.
[25] A.K. Naik, S.K. Tandan, D. Kumar, S.P. Dudhgaonkar, Nitric oxide and its modulators in chronic constriction injury-induced neuropathic pain in rats, Eur. J. Pharmacol. 530 (1) (2006) 59–69.
[26] G. Negi, A. Kumar, S.S. Sharma, Concurrent targeting of nitrosative stress–PARP pathway corrects functional, behavioral and biochemical deficits in experimental diabetic neuropathy, Biochem. Biophys. Res. Commun. 391 (1) (2010) 102–106.
[27] A.S. Jaggi, N. Singh, Exploring the potential of telmisartan in chronic constriction injury-induced neuropathic pain in rats, Eur. J. Pharmacol. 667 (1) (2011) 215–221.
[28] A. Kumar, R.K. Kaundal, S. Iyer, S.S. Sharma, Effects of resveratrol on nerve functions, oxidative stress and DNA fragmentation in experimental diabetic neuropathy, Life Sci. 80 (13) (2007) 1236–1244.
[29] J. Sedlak, R.H. Lindsay, Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent, Anal. Biochem. 25 (1968) 192–205.
[30] D. Bridges, S.W.N. Thompson, A.S.C. Rice, Mechanisms of neuropathic pain, Br. J. Anaesth. 87 (1) (2001) 12–26.
[31] M. Costigan, J. Scholz, C.J. Woolf, Neuropathic pain: a maladaptive response of the nervous system to damage, Annu. Rev. Neurosci. 32 (2009) 1.
[32] A. Janzade, S.B. Jameie, S. Choobchian, F. Nasirinezhad, Neuroprotective effect of coenzyme Q10 in chronic constriction injury-induced neuropathic pain in rat, Thrita 3 (1) (2014) e16607.
[33] X. Zhu, Q. Li, R. Chang, D. Yang, Z. Song, Q. Guo, C. Huang, Curcumin alleviates neuropathic pain by inhibiting p300/CBP histone acetyltransferase activity-regulated expression of BDNF and cox-2 in a rat model, PLoS One 9 (3) (2014) e91303.
[34] A. Maripuu, A. Bjorkman, I.M. Bjorkman-Burtscher, P. Mannfolk, G. Andersson, L.B. Dahlin, Reconstruction of sciatic nerve after traumatic injury in humans—factors influencing outcome as related to neurobiological knowledge from animal research, J. Brachial Plex. Peripher. Nerve Inj. 7 (1) (2012) 7.
[35] F. Li, C. Szabo, P. Pacher, G.J. Southan, O.I. Abatan, T. Charniauskaya, M.J. Stevens, I.G. Obrosova, Evaluation of orally active poly (ADP-ribose) polymerase inhibitor in streptozotocin-diabetic rat model of early peripheral neuropathy, Diabetologia 47 (4) (2004) 710–717.
[36] R. Baron, Mechanisms of disease: neuropathic pain—a clinical perspective, Nat. Clin. Pract. Neurol. 2 (2) (2006) 95–106.
[37] M.H. Ossipov, F. Porreca, Challenges in the development of novel treatment strategies for neuropathic pain, NeuroRx 2 (4) (2005) 650–661.
[38] F. Marchand, M. Perretti, S.B. McMahon, Role of the immune system in chronic pain, Nat. Rev. Neurosci. 6 (7) (2005) 521–532.
[39] C. Gueguen, B. Palmier, M. Plotkine, C. Marchand-Leroux, V.C. Besson, Neurological and histological consequences induced by in vivo cerebral oxidative stress: evidence for beneficial effects of SRT1720, a sirtuin 1 activator, and sirtuin 1-mediated neuroprotective effects of poly (ADP-ribose) polymerase inhibition, PLoS One 9 (2) (2014), e87367.
[40] S. Lupachyk, H. Shevalye, Y. Maksimchyk, V.R. Drel, I.G. Obrosova, PARP inhibition alleviates diabetes-induced systemic oxidative stress and neural tissue 4-hydroxynonenal adduct accumulation: correlation with peripheral nerve function, Free Radic. Biol. Med. 50 (10) (2011) 1400–1409.
[41] Z. Khalil, B. Khodr, A role for free radicals and nitric oxide in delayed recovery in aged rats with chronic constriction nerve injury, Free Radic. Biol. Med. 31 (4) (2001) 430–439.
[42] H.K. Kim, S.K. Park, J.-L. Zhou, G. Taglialatela, K. Chung, R.E. Coggeshall, J.M. Chung, Reactive oxygen species (ROS) play an important role in a rat model of neuropathic pain, Pain 111 (1) (2004) 116–124.
[43] A. Areti, V.G. Yerra, V.G.M. Naidu, A. Kumar, Oxidative stress and nerve damage: role in chemotherapy induced peripheral neuropathy, Redox Biol. 2 (2014) 289–295.
[44] X. Ba, N.J. Garg, Signaling mechanism of poly (ADP-ribose) polymerase-1 (PARP-1) in inflammatory diseases, Am. J. Pathol. 178 (3) (2011) 946–955.
[45] P.O. Hassa, M.O. Hottiger, The functional role of poly (ADP-ribose) polymerase 1 as novel coactivator of NF-κB in inflammatory disorders, Cell. Mol. Life Sci. 59 (9) (2002) 1534–1553.
[46] J.G. Lees, S.S. Duffy, G. Moalem-Taylor, Immunotherapy targeting cytokines in neuropathic pain, Front. Pharmacol. 4 (142) (2013) 1–4.
[47] L. Leung, C.M. Cahill, Review TNF-alpha and neuropathic pain—a review, J. Neuroinflammation 7 (27) (2010) 1–11.
[48] C. Sommer, M. Kress, Recent findings on how proinflammatory cytokines cause pain: peripheral mechanisms in inflammatory and neuropathic hyperalgesia, Neurosci. Lett. 361 (1) (2004) 184–187.
[49] A. Ellis, D.L.H. Bennett, Neuroinflammation and the generation of neuropathic pain, Br. J. Anaesth. 111 (1) (2013) 26–37.
[50] R. Janklek, P. Dubovi, I. Svizenska, I. Klusakova, Research bilateral changes of TNF-alpha and IL-10 protein in the lumbar and cervical dorsal root ganglia following a unilateral chronic constriction injury of the sciatic nerve, J. Neuroinflammation 7 (11) (2010).
[51] T. Doyle, Z. Chen, C. Muscoli, L.M. Obeid, D. Salvemini, Intraplantar-injected ceramide in rats induces hyperalgesia through an NF-kB-and p38 kinase-dependent cyclooxygenase 2/prostaglandin E2 pathway, FASEB J. 25 (8) (2011) 2782–2791.

[52] D. Siniscalco, C. Fuccio, C. Giordano, F. Ferraraccio, E. Palazzo, L. Luongo, F. Rossi, K.A. Roth, S. Maione, V. de Novellis, Role of reactive oxygen species and spinal cord apoptotic genes in the development of neuropathic pain, Pharmacol. Res. 55 (2) (2007) 158–166.
[53] T. Sun, W.G. Song, Z.J. Fu, Z.H. Liu, Y.M. Liu, S.L. Yao, Alleviation of neuropathic pain by intrathecal injection of antisense oligonucleotides to p65 subunit of NF-kB, Br. J. Anaesth. 97 (4) (2006) 553–558.
[54] S.A. Andrabi, G.K.E. Umanah, C. Chang, D.A. Stevens, S.S. Karuppagounder, J.-P. Gagn, G.G. Poirier, V.L. Dawson, T.M. Dawson, Poly (ADP-ribose) polymerase-dependent energy depletion occurs through inhibition of glycolysis, Proc. Natl. Acad. Sci. 111 (28) (2014) 10209–10214.
[55] S. Khan, O. Shehzad, J. Chun, Y.S. Kim, Mechanism underlying anti-hyperalgesic and anti-allodynic properties of anomalin in both acute and chronic inflammatory pain models in mice through inhibition of NF-kB, MAPKs and CREB signaling cascades, Eur. J. Pharmacol. 718 (1) (2013) 448–458.