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**RESEARCH ARTICLE**

# Attenuating Effect of Icariin, a Flavanoid in Partial Sciatic Nerve Ligation Induced Neuropathic Pain in Rats: An Evidence of Anti-Oxidative, Anti-Nociceptive and Neuroprotective Activity

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**ABSTRACT**

Neurodegeneration of the central and peripheral nervous system causes neuropathic pain. The present study has been investigated the attenuating role of Icariin (ICA) against partial sciatic nerve ligation (PSNL) induced peripheral neuropathy in rats. Adult male rats were divided into six groups with six rats in each group and partial ligation was performed on left sciatic nerve in group II to group VI; Sham control, PSNL control, Pregabalin (standard control), ICA 50 mg/kg/p.o, ICA 100 mg/kg/p.o and ICA 100 mg/kg alone (no exposure/ligation) groups. Oral administration of vehicle/pregabalin/ICA was given once daily for 21 consecutive days based on groups. Various nociceptive behavioral parameters were investigated by assessing foot deformity score, motor co-ordination test, cold allodynia, radiant heat hyperalgesia, exploratory behavior and spontaneous locomotor activity which were done on $0^{th}$, $7^{th}$, $14^{th}$ and $21^{st}$ days. Biochemical tests like superoxide dismutase (SOD), calcium (Ca2+), Catalase (CAT), reduced glutathione (GSH), glutathione reductase (GR) and histopathological changes were measured in sciatic nerve homogenate. Treatment with ICA and pregabalin significantly ($p$<0.001) attenuated the PSNL induced neuropathic pain in dose dependent manner in all nociceptive pain tests and biochemical oxidative stress markers. Therefore, it is concluded that ICA has promising neuroprotective action against PSNL induced neuropathic pain due to its anti-oxidant and anti-nociceptive effects.

**Keywords:** Icariin, Partial sciatic nerve ligation, Neuropathic Pain

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**C O N T E N T S**



## 1. Introduction

Neuropathic pain (NP) is defined as "Pain caused by a lesion or disease of the somatosensory system" or "Pain initiated or caused by a primary lesion, dysfunction, or transitory perturbation of the peripheral or central nervous system" [1]**.** It is often caused due to injury of peripheral, central neural dysfunction along with etiological causes which include metabolic diseases, ischemia, infections, neurodegenerative diseases and drugs [2]. This can be characterized by burning, shooting and stabbing pain sensation; The pain stimulus is exaggerated even more due to non noxious and noxious stimuli. In India, approximately 4 million people are affected with acute and chronic pain [3]. Different studies have been proved that the NP is partially maintained by sympathetic nervous system. However, patients are non-responsive to pharmacological treatment. Many neuropathic pain patients had reported both positive signs such as paresthesias, spontaneous pain, dysesthesias and allodynia along with negative abnormalities such as hyperpathia, hyperesthesia, and hyperalgesia.

Pharmacological treatment such as anticonvulsants, antidepressants, opioids, selective serotonin reuptake inhibitors [SSRIs], serotonin–norepinephrine reuptake inhibitors [SNRIs] and analgesics are used for treatment of NP [4]. Partial Sciatic Nerve Ligation (PSNL) model was the most widely accepted and established model to induce NP, as there is a significant injury to a large set of non-myelinated and myelinated axons due to partial ligation causing partial nerve injury [4, 5]. NP can be analyzed by the changes in behavior of mechanical, thermal allodynia and spontaneous pain thresholds [6]. Even though many advances in microsurgical techniques, molecular biology along with tissue engineering procedures for peripheral nerve repair was increased drastically [7], clinical management of neuropathic pain is very challenging due the heterogeneity of its etiologies, symptoms and underlying mechanisms. It was known the oxidative damage and inflammation plays an important role on the nerve damage, antioxidants were seen as an efficient means to combat the damages caused due to reactive oxygen species making them protective agents in the management of neurodegenerative diseases [8, 9]. ICA is obtained from *Epimedium brevicornum Maxim,* belongs to *"Berberidaceae"* family. It is also known as "Horny Goat Weed" or "Yin Yang Huo", in Chinese traditional medicine and utilized for several centuries in order to treat a different kind of human illness [10]. Recently, studies focused on this plant has suggested that Icariin (ICA), a flavonoidal glycoside is a metabolically active phytoconstituent of Epimedium that can be used for a wide variety of treatments due to its antioxidant and anti-nociceptive properties [11]. There are several studies suggesting that the ICA can promote muscle function recovery after injury to spinal cord in rats [12]. In this study, we evaluated the neuroprotective study of ICA against PSNL induced nociceptive behavioral, biochemical and histological changes in rats.

**Induction of peripheral neuropathic Pain**

The rats were anesthetized with ketamine 100 mg / kg and 10 mg /kg xylazine i.m. briefly, the rats were placed in prone position. Left thigh was incised and a cut was made directly through the biceps femoris to expose the 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 any ligation. Nociceptive threshold tests were assessed at every 7 day intervals from 0th day to 21 consecutive days [15, 16, 17].

## 2. Materials and Methods

**Drugs and Chemicals:** Icariin (ICA) was procured from S.V. Agro Foods, Maharashtra, India. All reagents used in this study were procured from Merck, Sigma-Aldrich, S.D. Fine were of analytical grade.

**Animals**

Wister Albino Male rats weighing approximately 150-200 grams were selected in this study to avoid estrous cycle fluctuations. Animals were maintained at 24±1 °C, with relative humidity of 45–55% and 12:12 h dark/light cycle and given free access to the food (Agro Corporation Private Ltd., Bangalore, India). These rats were then further divided into six groups with six rats in each group. Animals were acclimatized under laboratory conditions for 2 weeks prior to the experimentation [13, 14]. The study was carried out with prior approval from Institutional Animal Ethical Committee No. BRWN/IAEC/2014 dated 20/3/2014.

**Induction of peripheral neuropathic Pain:** The rats were anesthetized with thiopental sodium (35 mg/kg, i.p.). Left thigh was incised and a cut was made directly through the biceps femoris to expose the 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 any ligation. Nociceptive threshold tests were assessed at every 7 day intervals from $0^{th}$ day to 21 consecutive days [15, 16, 17].

**Experimental Study Protocol:** Rats were randomly divided into six groups of six animals in each group in which group-I (Sham control) and group -II (PSNL control) received vehicle only, group -III received Pregabalin 10 mg/kg orally (standard control), group IV and V received ICA 50 mg/kg and 100 mg/kg p.o respectively, group VI received ICA 100 mg/kg alone (without surgery). The animals underwent PSNL surgery except group I and group VI animals. All the animals were received their respective

treatment for a period of 21 days. Behavioral changes were assessed on $0^{th}$, $7^{th}$, $14^{th}$ and $21^{st}$ day intervals.

**Assessment of Nociceptive Behavioral Parameters**

***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: 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 [18].

***Cold allodynia test***

Cold allodynia test was performed as per the method described by Naik et al., [19]. In this method, terminal part of the rat tail was gently submerged in ice cold water (4 ± $1^0$C) in a beaker. The tail 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, [20]. Briefly, rats were placed individually on the rotating rod (25 rpm). The time taken by the rat to fall off from the rod was recorded.

***Radiant heat hyperalgesia test***

Radiant heat hyperalgesia test was evaluated by using a glass bottom enclosure a radiant heat source is positioned under the animal and is aimed at the left hind paw on the plantar surface. Cut off time was 15 seconds. The time taken for withdrawal of the left hind paw from heat stimulus was recorded. [21, 22]

***Test for Exploratory behavior***

Exploratory behavior was assessed by using Hole Board apparatus. Hole board consists of 16 holes. The rats were placed on the edge of the hole board for six minutes (2 mins for acclimatization) and remaining 4 minutes were recorded for number of head dipping for each rat was recorded [23].

***Test for Spontaneous locomotor activity***

Spontaneous locomotor activity was assessed by using UGO Basile digital Photoactometer .This test was employed to assess the effect of drug treatment on spontaneous motor activity. One hour after the respective treatment, animals are placed individually in Photoactometer and 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. An interruption of photocell beam occurs due to locomotor activity of rat were recorded by digital counter [24, 25, 26].

**Assessment of Biochemical Changes**

On $21^{st}$ day, 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 used for the estimation of levels of superoxide dismutase (SOD), Nitric oxide (NO), total calcium, catalase (CAT), reduced glutathione (GSH), glutathione reductase (GR), glutathione peroxidase (GPx), myeloperoxidase (MPO) and Tumor necrosis factor – α (TNF- α).

***Estimation of superoxide dismutase levels***

SOD activity was measured according to the method of Misra and Fridovich, [27] 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.

***Measurement of nitric oxide levels:*** To 100µl of brain supernatant, 500µl of Greiss reagent (1:1 solution of 1% sulphanilamide in 5% phosphoric acid and 0.1% naphthylamine diamine dihydrochloric acid in water) was added and absorbance was measured at 546nm [28]. Nitrite concentration was calculated using a standard curve of sodium nitrite and expressed as µmol of nitrite/mg protein.

***Measurement of total calcium levels***

The total calcium levels were measured according to manufacturer's instructions using commercially available kit (Span Diagnostics Ltd., India).

***Measurement of Catalase (CAT) levels***

Catalase levels was measured by a slightly modified version of Aebi, [29]. Briefly, 100 µl of supernatant was added to 10 µl of 100% ethanol and placed in ice bath for 30 min. The tubes were allowed to attain room temperature followed by the addition of 10 µl of Triton X-100. To 50 µl of mixture, 200 µl of phosphate buffer and 250 µl of 0.066 M $H_2O_2$ (in phosphate buffer) was added. The decrease in optical density was measured at 240 nm for 60 sec in UV Spectrophotometer and expressed in terms of moles of $H_2O_2$ degraded/mg protein/min.

***Measurement of reduced glutathione (GSH) content***

Reduced glutathione content were measured according to the method of Ellman, [30]. Briefly, 0.75ml of supernatant was mixed with 0.75ml of 4% sulphosalicylic acid and then centrifuged at 1,200rpm for 5min at $4^0$C. From this 0.5ml of supernatant was taken and added to 4.5ml of 0.01M DTNB, and the yellow color developed was read Spectrophometrically at 412nm immediately. The GSH content was calculated as nmol GSH/mg protein.

***Measurement of glutathione reductase (GR) levels***

GR activity was estimated according to the method of Calberg and Mannervick, [31]. The reaction mixture consisted of 0.05 ml of supernatant, phosphate buffer (0.1M, pH 7.4), 0.1 mM NADPH, 0.5 mM EDTA, 1 mM oxidized glutathione in a total volume of 1 ml. The enzyme activity was quantified by measuring the disappearance of NADPH at 340 nm and was expressed as nmol NADPH oxidized/min/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., [32]. Nerve sections were analyzed qualitatively under light microscope for axonal degeneration.

**Statistical analysis**

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

## 3. Results and Discussion

**Nociceptive Tests:**

***Effect of ICA on Foot deformity score:*** Upon PSNL ligation, foot deformity was observed in all animals. The rats with induced neuropathy developed abnormal gait and posture. The foot was ventroflexed, with the toes held tightly together and rats were unwilling to place weight on the injured foot. Foot positioning and toe spread rating was significantly ($p<0.001$) different between sham control and experimental groups. Treatment with ICA (50,100mg/ kg/p.o) and standard Pregabalin significantly ($p<0.001$) reduced the foot deformation score compared PSNL group (Figure 1).

***Effect of ICA on Cold Allodynia:*** Cold Allodynia was assessed by tail withdrawal latency time. In PSNL ligated group, tail withdrawal latency time was significantly ($p<0.001$) reduced on 7$^{th}$, 14$^{th}$ and 21$^{st}$ days when compared to sham control group. Whereas treatment with ICA 50,100mg/kg/p.o significantly ($p<0.05$) ($p<0.001$) increased tail withdrawal latency time in dose dependent manner on different intervals compared to PSNL group. Comparable significant increase was observed in PREG 10mg/kg/p.o treated group ($p<0.001$). Sham operated group did not show any change in withdrawal latency time. (Figure 2).

***Effect of ICA on Radiant heat hyperalgesia***

Radiant heat hyperalgesia was assessed by paw withdrawal latency time. PSNL ligated group showed significant decrease in the paw withdrawal latency time on 7$^{th}$, 14$^{th}$ and 21$^{st}$ days ($p<0.001$). Administration of ICA 50, 100 mg/kg/p.o attenuated PSNL induced decrease in paw withdrawal time significantly ($p<0.01$) ($p<0.001$) on 14$^{th}$ and 21$^{st}$ days when compared to PSNL group. Pregabalin 10 mg/kg/p.o treated group significantly ($p<0.001$) attenuated the paw withdrawal latency time on 7$^{th}$, 14$^{th}$ and 21$^{st}$ days as compared to PSNL group (Figure 3).

***Effect of ICA on Exploratory Behavior***

Treatment with ICA significantly attenuated PSNL induced change in exploratory behavior in a dose dependent manner assessed by no. of head dippings. In PSNL ligated group, number of exploratory movements was significantly reduced on 7$^{th}$, 14$^{th}$ and 21$^{st}$ days compared to sham control groups ($p<0.001$). Rats subjected to ICA 50, 100 mg/kg/p.o and PREG 10mg/kg/p.o showed increased number of exploratory movements on 7$^{th}$, 14$^{th}$ and 21$^{st}$ days compared to PSNL groups (Figure 4).

***Effect of ICA on Spontaneous locomotor activity***

PSNL ligated group showed significant ($p<0.001$) decrease in spontaneous locomotor activity (No. of counts) when compared to sham control group. Treatment with ICA (50, 100 mg/kg/p.o) and PREG 10 mg/kg groups showed significant increase on 21$^{st}$ day ($p<0.001$) when compared to PSNL groups (Figure 5).

***Effect of ICA on Motor coordination***

Administration of the ICA 50, 100 mg/kg/p.o and PREG 10 mg/kg/p.o significantly attenuated PSNL induced decrease in motor performance, assessed by time spent on revolving rod in a dose-dependent manner (Figure 4). when compared to PSNL group (Figure 6).

**Biochemical studies**

***Effect of ICA on Superoxide dismutase (SOD) levels***

SOD levels were significantly decreased ($p<0.001$) in PSNL group as compared to sham control group. Treatment with ICA (50,100mg/kg/p.o) groups significantly ($p<0.01$) ($p<0.001$) attenuated the PSNL induced increase the SOD levels when compared to PSNL control group in a dose dependent manner. Comparable Significant increase ($p<0.001$) in SOD levels were found in PREG (10mg/kg/ p.o) treated group (Figure 7).

***Effect of ICA on Nitric oxide (NO) levels***

Nitric oxide levels were significantly increased ($p<0.001$) in PSNL group as compared to sham control group which indicates the neuropathic pain induction. Treatment with ICA (50, 100mg/kg/p.o) groups significantly ($p<0.01$) ($p<0.001$) attenuated the PSNL induced decrease in the NO levels when compared to PSNL control group in a dose dependent manner. Comparable Significant decrease ($p<0.001$) in NO levels were found in PREG (10mg/kg/p.o) treated group (Figure 8).

***Effect of ICA on Calcium levels***

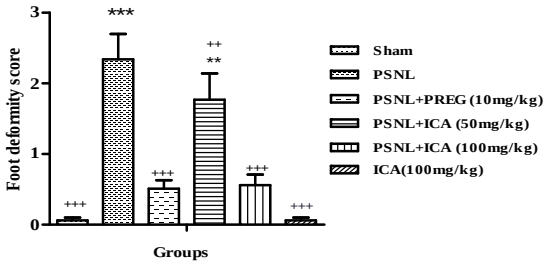
Calcium levels were significantly increased ($p<0.001$) in PSNL group when compared to sham control group which indicates the induction of neuropathic pain. Treatment with ICA (50,100mg/kg/p.o) and PREG 10 mg/kg/p.o groups significantly ($p<0.01$) ($p<0.001$) prevented the PSNL induced decrease in the calcium levels when compared to PSNL control group in a dose dependent manner. (Figure 9).

***Effect of ICA on GSH, GR and CAT levels***

The levels of GSH, GR and CAT were significantly decreased ($p<0.001$) in PSNL group as compared to sham control group. Treatment with ICA (50,100mg/kg/p.o) groups significantly ($p<0.01$) ($p<0.001$) attenuated ligation induced increased in the levels of GSH (Fig 10), GR (Fig 11) and CAT (Fig 12) dependently as compared to PSNL group animals. Comparable Significant increase ($p<0.001$) were found in PREG (10mg/kg/p.o) treated group.

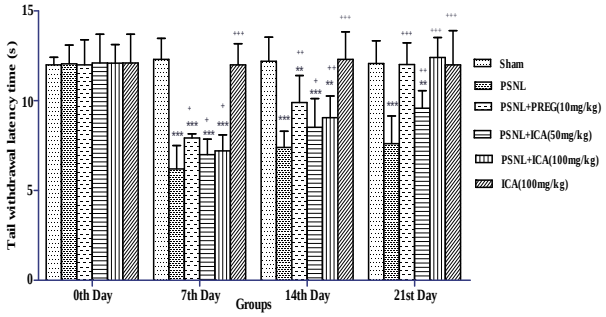
***Effect of ICA on Histopathology studies:*** In PSNL group animals, significant histopathological changes were observed in sciatic nerve. Degeneration of normal axons were found which is evident by decreased number of Schwann cells and the presence of high vacuolization as compared to control group. Treatment with ICA (50,100mg/kg/p.o) groups significantly ameliorated histopathological changes in dose dependent manner as compared to PSNL group animals. However, PREG (10mg/ kg/p.o) treated group animals also showed significant recovery as that of ICA 100mg/kg/p.o as compared to control group (Figure 13).

**Figure: 1 - Effect of ICA on Foot deformity score in PSNL rats**



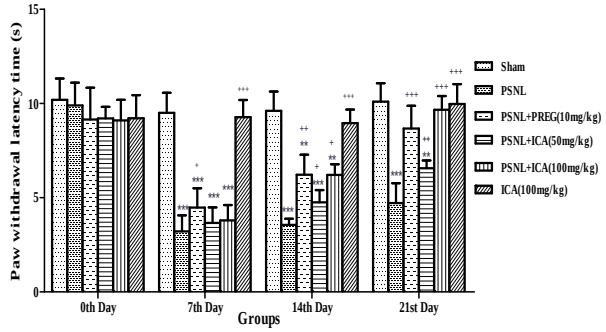
**Values expressed as Mean ± SEM (n=6);**
****, ***, (p<0.01), (p<0.001) Vs Sham control group;**
**++, +++, (p<0.01), (p<0.001) Vs PSNL control group**

**Figure: 2 - Effect of ICA on Cold Allodynia in PSNL rats**



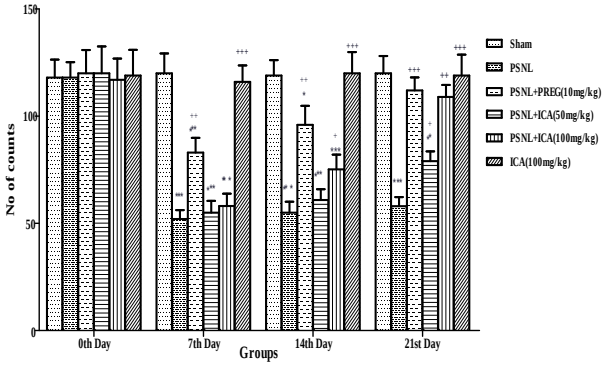

**Values expressed as Mean ± SEM (n=6); **, ***, (p<0.01), (p<0.001) Vs Sham control group +, ++, +++, (p<0.05), (p<0.01), (p<0.001) Vs PSNL control group**

**Figure: 3 - Effect of ICA on Radiant heat hyperalgesia in PSNL rats**



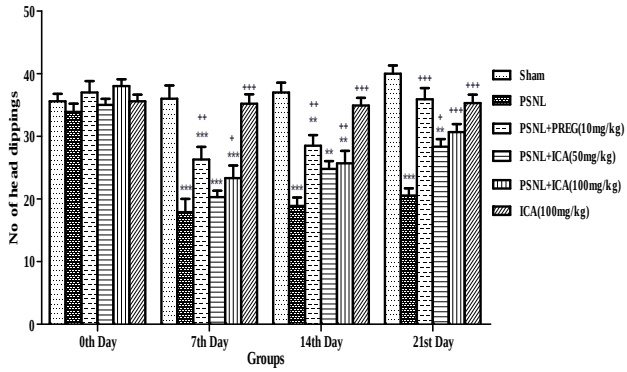

**Values expressed as Mean ± SEM (n=6);**
****, ***, (p<0.01), (p<0.001) Vs Sham Control group**
**+, ++, +++, (p<0.05), (p<0.01), (p<0.001) Vs PSNL Control group**

**Figure: 4 - Effect of ICA on Spontaneous locomotor activity in PSNL rats**



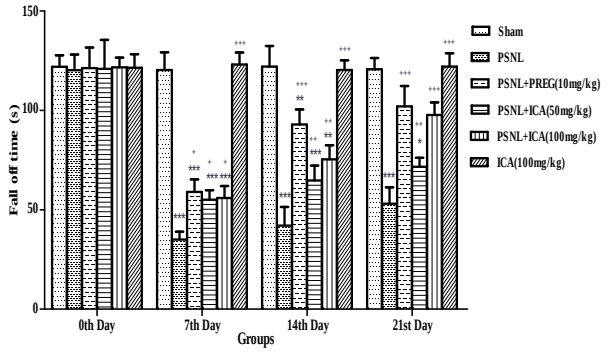

**Values expressed as Mean ± SEM (n=6);**
***, **, ***, (p<0.05), (p<0.01), (p<0.001) Vs Sham Control group**
**+, ++, +++, (p<0.05), (p<0.01), (p<0.001) Vs PSNL Control group**

**Figure: 5 - Effect of ICA on exploratory behavior in PSNL rats**



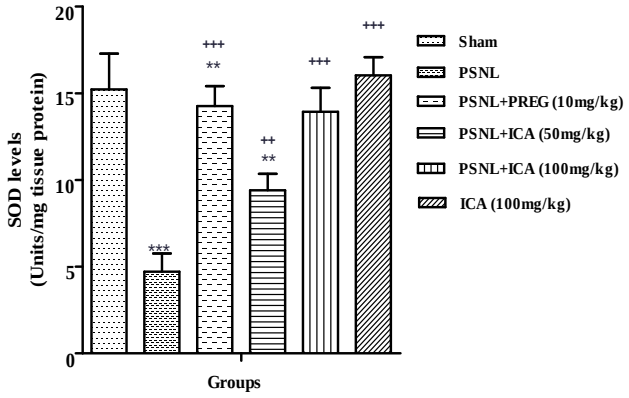

**Values expressed as Mean ± SEM (n=6);**
****, ***, (p<0.01), (p<0.001) Vs Sham control group**
**+, ++, +++, (p<0.05), (p<0.01), (p<0.001) Vs PSNL control group**

**Figure: 6 - Effect of ICA on Motor coordination in PSNL rats**



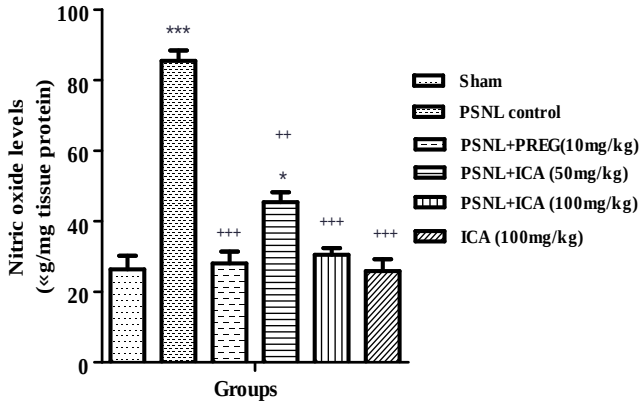

**Values are expressed as Mean ± SEM (n=6);**
***,**, ***, (p<0.05), (p<0.01), (p<0.001) Vs Sham control group**
**+, ++, +++, (p<0.05), (p<0.01), (p<0.001) Vs PSNL control group**

**Figure: 7 - Effect of ICA on SOD levels in PSNL rats**



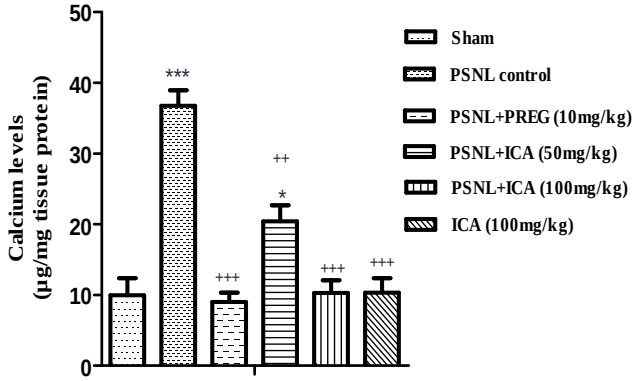

**Values expressed as Mean ± SEM (n=6); **, ***, (p<0.01), (p<0.001) Vs Sham control group**
**++, +++, (p<0.01), (p<0.001) Vs PSNL control group**

**Figure: 8 - Effect of ICA on Nitric oxide (NO) levels in PSNL rats**



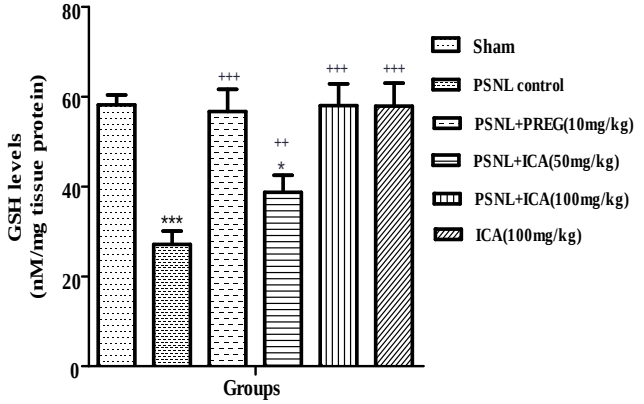

**Values expressed as Mean ± SEM (n=6); *, ***, (p<0.05), (p<0.001) Vs Sham control group ++, +++, (p<0.01), (p<0.001) Vs PSNL control group**

**Figure: 9 Effect of ICA on calcium levels in PSNL rats**



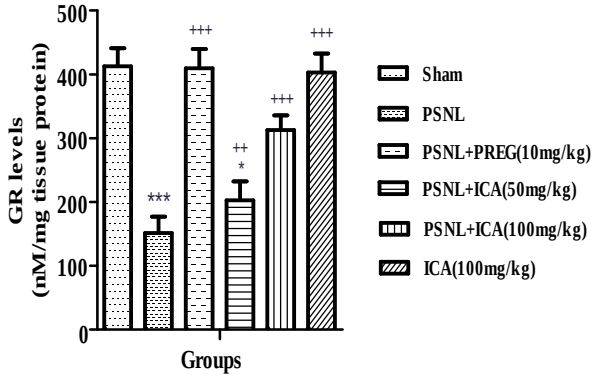

**Values expressed as Mean ± SEM (n=6); *, ***, (p<0.05), (p<0.001) Vs Sham control group ++, +++, (p<0.01), (p<0.001) Vs PSNL control group**

**Figure: 10 Effect of ICA on GSH levels in PSNL rats**



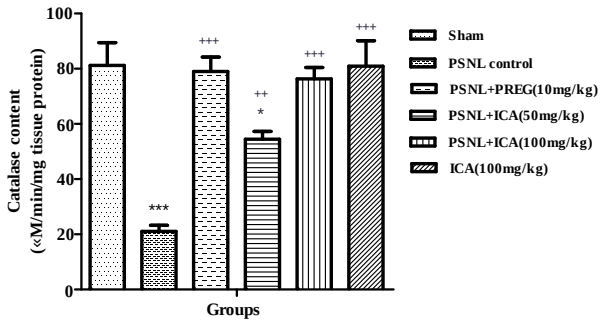

**Values expressed as Mean ± SEM (n=6); *, ***, (p<0.05), (p<0.001) Vs Sham control group ++, +++, (p<0.01), (p<0.001) Vs PSNL control group**

**Figure: 11 - Effect of ICA on GR levels in PSNL rats**




**Values expressed as Mean ± SEM (n=6); *, ***, (p<0.05), (p<0.001) Vs Sham control group ++, +++, (p<0.01), (p<0.001) Vs PSNL control group**

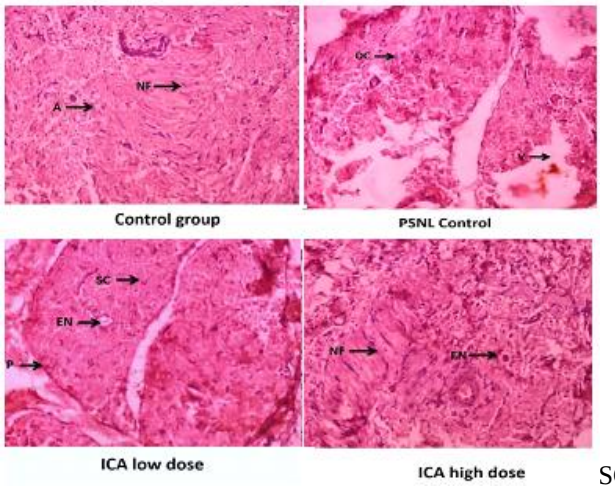
**Figure: 12 - Effect of ICA on Catalase levels in PSNL rats**




**Values expressed as Mean ± SEM (n=6); *, ***, (p<0.05), (p<0.001) Vs Sham control group ++, +++, (p<0.01), (p<0.001) Vs PSNL control group**

**Histopathological studies:**

**Figure: 13- Effect of ICA on Histopathological changes in Sciatic Nerve.**




SC- Schwann cell nuclei, NF- Nerve fibers, Ax- Axon, DC- degenerative changes, V- Vacuolation, EN – Endoneurium, P – Perineurium

**Discussion**

Neuropathic pain is a common and debilitating problem which challenges health-care professionals and researchers. There is no curative conventional treatment for neuropathic pain despite of available of drugs in large number. Nowadays, more attention has been focused on the herbal plant research and their active compounds in the field of drug discovery due to their lower complications and fewer side effects than synthetic drugs [33]. In this study, we evaluated herbal active compound Icariin (ICA), a flavanoidal glycoside obtained *Epimedium brevicornum Maxim*, belongs to "*Berberidaceae"* family. It is also called as "Horny Goat Weed" or "Yin Yang Huo", is a classic tonic agent in traditional Chinese herbal medicine. ICA was reported to have antioxidant activity [34-36]. Hence, the present study was investigated to know the protective role of Icariin against partial sciatic nerve ligation (PSNL) induced peripheral neuropathy in rats.

In neuropathic pain, there is central sensitization of neurons which may damages motor as well as sensory fibers, where NP influences the quality of life through neurobehavioral changes [37]. The sensory and motor damage is characterized through assessment of foot deformity score, cold allodynia, motor coordination test, radiant heat hyperalgesia, exploratory behavior, spontaneous locomotor activity [38-42, 23]. Evaluation of foot deformity score reveals the degree of injury and recovery based on its score. Foot positioning and toe spread are useful in assessing the locomotor and behavioral movements. Rats treated with PREG, ICA (100mg/kg/p.o) both showed least score for foot deformation, indicating its protective action against NP induced foot deformation. NP is often associated with the appearance of abnormal sensory signs, such as allodynia and hyperalgesia [43]. PSNL showed significant decrease in pain threshold for cold allodynia test and radiant heat hyperalgesia. PREG and ICA treatment significantly prevented PSNL induced decrease in pain threshold. In our study, we observed neurobehavioral changes with partial sciatic nerve ligation which was attenuated with ICA treatment indicating its potential. Experimental evidences suggested that an increased tissue total calcium levels has been demonstrated during neuropathic pain [44,45,23]. The increased calcium levels activates the secondary messengers i.e., calpain and calmodulin which alters the homeostasis of nervous system through axonal degeneration and stability [46]. Inconsistent with these evidences, in our study, the PSNL group showed increased levels of calcium ions which was significantly prevented with the ICA and Pregabalin treatment [47, 39]. Several studies evidenced that free radical and calcium mediated oxidative stress play a major role in the pathogenesis of neurodegenerative diseases including neuropathic pain [48, 49]. Primary injury to sciatic nerve discharges ectopic firing in the spinal cord which may increase mitochondrial respiration as well as intracellular calcium, consequently lead to increased ROS production. Excess ROS build up in glial cells which then leak to produce neuronal dysfunction and pain. It is likely that ROS triggers second messengers involved in central sensitization of dorsal horn cells which results in chronic pain [50, 51]. Experimental evidences suggested that herbal medicinal plants and its phytocompounds have been reported as neuroprotectants through attenuation of NP induced oxidative stress [45,36,39]. The results from our clearly demonstrated that ICA attenuated PSNL induced increased LPO levels and decreased SOD, GSH, CAT levels which was confirmed with the histological studies.

## 4. Conclusion

From our results, it is evident that ICA may be a promising neuroprotective agent against PSNL induced neuropathic pain due to its anti-oxidant and anti-nociceptive actions.

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