Physiology and Pharmacology 27 (2023) 392-402
Original Article

Neuroprotective effect of *Acorus calamus* Linn. extract on a rat model of chronic constriction injury of median nerve-induced peripheral neuropathy

Shubhechha Bansod, Likhit Akotkar, Subhash Bodhankar, Urmila Aswar*

Department of Pharmacology, Poona College of Pharmacy, Bharati Vidyapeeth (Deemed to be University) Pune, India

ABSTRACT

Introduction: Acorus calamus Linn. from the Acoraceae family exhibits several benefits in neurological disorders but has not been studied for chronic constriction injury (CCI) of median nerve induced neuropathic pain. Damage to median nerve leads to work-related musculoskeletal disorders (WMSDs). this study aimed to assess the effects of the ethanolic root extract of Acorus calamus (EAC) on CCI-induced neuropathic pain and WMSDs in rats.

Methods: Animals were randomly divided into 7 groups of 8 animals each. Group 1. Normal control, 2. Sham control, 3. CCI, 4. CCI+ vehicle (CMC), 5. CCI+gabapentin (50 mg/kg), 6. CCI+EAC (20 mg/kg), 7. CCI+EAC (40 mg/kg). On day 0, rats were subjected to the surgical procedure of exposure and ligation of the median nerve-produced CCI at the forearm level. Pain-sensitive tests (i.e., hot plate test, Randall Selitto test), and functional analysis (i.e., walking track) were performed. Total protein, lipid peroxidation, and histopathological changes were also estimated.

Results: CCI significantly increased thermal and mechanical hyperalgesia, raised median functional index (walking track analysis), and induced biochemical and histological disruptions. Oral administration of EAC (40 mg/kg) and gabapentin (50 mg/kg) notably lowered CCI-induced nociceptive pain threshold, improved median nerve functional index, and mitigated tissue histological alterations.

Conclusion: EAC has been found to decrease CCI-induced neuropathic pain of the median nerve. Its mechanisms likely involve neuroprotective, antioxidant, and anti-inflammatory properties.

Keywords:
Acorus calamus
Median nerve injury
Nerve functional index
Neuroprotection
Neuropathic pain
Walking track analysis

Introduction

Peripheral neuropathy (PN) occurs when nerves that carry messages from the brain and spinal cord to the rest of the body are damaged or diseased (Chen et al., 2010). PN can be acute or chronic and it can also be reversible or permanent. Various types of neuropathies include motor neuropathy, sensory neuropathy, autonomic nerve neuropathy, and combination neuropathies. Motor

* Corresponding author: Urmila Aswar, urmila.aswar@bharatividyapeeth.edu
Received 9 July 2022; Revised from 23 November 2022; Accepted 31 December 2022

Citation: Bansod S, Akotkar L, Bodhankar S, Aswar U. Neuroprotective effect of *Acorus calamus* Linn. extract on a rat model of chronic constriction injury of median nerve-induced peripheral neuropathy. Physiology and Pharmacology 2023; 27: 392-402. http://dx.doi.org/10.61186/phypha.27.4.392

neuropathy results from damage to nerves that control muscles and movement in the body, such as restricted movement of hands and/or using fingers (Fujiwara et al., 2017). PN is one of the most common complaints of people referred to physicians for proper diagnosis. Median nerve dysfunction is a type of PN that intrude the movement of or sensation in the hands leading to work-related musculoskeletal disorders (WMSDs). According to prior research, there are three main pathophysiological mechanisms involved in the development of WMSDs that is, central nervous system (CNS) reorganization, tissue injury, and tissue reorganization. In patients with WMSDs, any of these mechanisms, alone or in combination, may induce pain, discomfort, and/or loss of function contributing to substantial medical costs and productivity loss (Krishnan et al., 2021; Silverstein et al., 2004). *Acorus calamus* Linn. (Acoraceae) is a traditional Indian medicinal herb that is also known as "Vacha" in Sanskrit, which means "clear speech" because it enhances intelligence and expressiveness. It's used to treat a variety of illnesses, including neurological, gastrointestinal, respiratory, metabolic, kidney, and liver problems. It is useful in treating psychological imbalances, mood disorders, and as antispasmodic for seized muscles (Rajput et al., 2014). It contains active coumponds like saponins, glycosides, flavonoids, tannins, polyphenolic compounds, mucilage, and volatile oil which are known for anti-inflammatory, and antioxidant activity (Muthuraman and Singh 2012; Shukla et al., 2002). The complications arising from median nerve constriction, including bicipital aponeurosis and carpal tunnel syndrome due to compression within the carpal tunnel, pose significant challenges. Current treatments for these complications involve a spectrum of approaches such as non-steroidal anti-inflammatory drugs, opioids, surgical procedures, exercise, and the use of temperature therapies like heat or cold. However, these methods often fall short in effectively alleviating neuropathic pain (Ortega-Álvaro et al., 2012). Managing neuropathic pain proves to be intricate, and the response to medications remains unsatisfactory. Additionally, these treatments may bring along adverse effects such as sedation, dry mouth, blurred vision, weight gain, urinary retention, dizziness, and peripheral edema (Baron et al., 2010). The ligation of the sciatic nerve has been shown to induce several neuropathic pain symptoms such as mechanical allodynia, thermal hyperalgesia, and an increase in oxidative stress markers like lipid peroxidation and nitrite levels. Additionally, this leads to a reduction in antioxidant enzyme levels such as catalase, superoxide dismutase, and glutathione peroxidase within the sciatic nerve tissue (Zulazmi et al., 2015). Traditionally, in rat models studying PN pain, sciatic nerve injury has been predominantly used. However, the current study opts to induce PN using the median nerve due to various advantages this rat model presents over the sciatic nerve model. These benefits include a decreased incidence of joint contractures and auto-mutilation in the affected limb, easier dissection due to minimal muscle mass coverage, quicker recovery compared to the sciatic nerve model, and the feasibility of using the ulnar nerve as a graft for median nerve repair (Bertelli et al., 1996). Opting for the median nerve neuropathy model holds significance considering the high susceptibility of upper limb nerves to injuries in humans. Given that median nerve injuries commonly affect laborers and workers, leading to peripheral CCI, we've chosen this model for our study. Ethanolic extract of *Acorus calamus* (EAC) has been reported earlier for its effects on CCI of sciatic nerve in peripheral neuropathy (Muthuraman and Singh 2011). To evaluate the effectiveness of EAC in addressing CCI-of median nerve, we conducted an investigation using a median nerve neuropathy model in Wistar rats.

Material and Methods

Drugs and Chemicals

Thiopental sodium (Thiosol; Neon Laboratories Ltd, India), and Gabapentin, (Neurontin; Pfizer, USA) were purchased, while surgical supplies including 7-0 nylon suture and other chemicals used for biochemical estimation were obtained from local vendors.

Authentication of a Dried Sample of Acorus calamus

The dried and pressed samples of the whole *Acorus calamus* plant were obtained from the local market and authenticated at the Regional Ayurveda Research Institute, Pune (Accession No. 14925).

Preparation of Ethanolic Extract of Acorus calamus

The whole *Acorus calamus* plant was dried and pressed to eliminate moisture. The roots were then crushed into fine powder. This powder was used for extraction using the maceration technique. A sample weight of 250 g of *Acorus calamus* root powder was placed inside

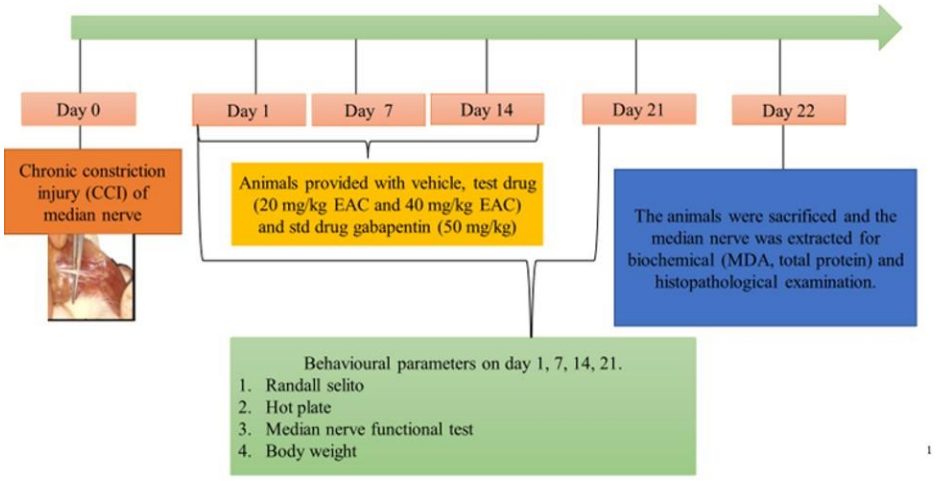

FIGURE 1. Schematic representation of study protocol.

an airtight jar with an extraction solvent consisting of 90% ethanol and 10% water. The mixture was regularly shaken for 24 hours within an airtight container. The resulting hydroalcoholic extract was concentrated by removing ethanol using a rotary evaporator at temperatures below 40°C. Finally, the concentrated extracts were dried by a mini spray dryer equipped with a 0.7 mm diameter nozzle (Yousuf et al., 2020).

Induction of PN by CCI of Median Nerve

Female Wistar rats, twelve weeks old, were anesthetized using sodium thiopental at a dosage of 35mg/kg intraperitoneally (ip). The hair over the medial aspect of the right forearm was removed, and an incision was made using a scalpel. Following the division of the brachial fascia, the median nerve below the muscle was identified. Briefly, one-third to one-half of the right median nerve was loosely ligated by tying 4 loose ligatures at a distance of 1 mm using silk suture, and the skin wound was stitched close using 4-0 nylon suture. Sham surgery was performed on age-matched animals, involving exposure of the right median nerve without ligation. The muscle layers were closed with 4-0 nylon sutures, followed by skin closure (Bennett and Xie 1988).

Experimental Design

After a one-week recovery period, the animals were divided randomly into 7 groups, each comprising 8 animals. Group 1- Normal control: Animals not subjected to CCI, given only food and were *ad libitum*. Group 2- Sham control: Sham animals with the right median nerve exposed but without ligation, provided with food and water *ad libitum*. Group 3- CCI control: Animals that underwent CCI surgery to ligate the right median nerve, administered with vehicle. Group 4- Vehicle control: CCI surgery was performed on animals to ligate the right median nerve, receiving only the vehicle (CMC 0.5%). Group 5- Standard treatment: Animals that underwent surgery to ligate the right median nerve, treated with gabapentin (50 mg/kg) Group 6-Test treatment (low dose): Surgery performed to ligate the right median nerve, treated with EAC (20 mg/kg) Group 7- Tzest treatment (high dose): Surgery to ligate the right median nerve, treated with EAC (40 mg/kg). All the drugs were only administered once daily from day 1 to day 21 after surgery (Figure 1).

Behavioral Parameters

Randall Selitto

The paw withdrawal capacity of the animal was evaluated using Randall Selitto apparatus (IITC Life Science, USA). The animals' paw was placed on to the designated region, and weight was applied gradually through the scale. The measurement recorded was the point at which the animal withdrew its leg towards its body. The scale is calibrated starting from 1, equating to 32 grams (1=32 g), and subsequent readings were analyzed in grams (Randall et al., 1957).

Hot Plate Test

The hot plate test was conducted to detect the thermal allodynia following the induction of neuropathic pain.

The hot plate was kept at a constant temperature of 55° C. Rats were placed on the hot plate, and their heat tolerance were observed. any sign of licking or jumping were considered as a response. Each experimental trial was separated by a 5-minute interval, and a 15-second cut-off time was implemented to prevent tissue damage. Animals exhibiting baseline latencies higher than 20 seconds were excluded from the study (Eddy and Leimbach 1953).

Walking Track Analysis

The median functional index of the animals was assessed by having them walk on a narrow track with a food palette placed at the track's end, and their footprints were recorded. Several parameters were measured from both the experimental (E) and normal (N) sides, including print length (PL) - the distance from the heel to the second toe, print angle (PA) - the angle of the foot from the midline track, toe spread (TS) - the distance from the first to the fourth toe, and intermediary toe spread (ITS) - the distance between the second and third toe.

Various factors were calculated using these measurements: Print length factor (PLF)= (EPL-NPL)/NPL, Toe spread factor (TSF)= (ETS-NTS)/NTS, and Intermediary toe spread factor (ITF)= (EIT-NIT)/NIT.

The final formula of Functional index (FI)= -38.3 × PLF + 109.5 ×TSF + 13.3 × ITF – 8.8 (Bain et al., 1989).

Biochemical Estimations

After the completion of behavioral assessments, all animals were euthanized on the 22nd day. Sections of the median nerve were promptly isolated for the evaluation of malondialdehyde (MDA) levels and total protein content.

Thiobarbituric acid test (TBA)

The assessment involves the reaction between malondialdehyde (MDA), an end product of lipid peroxidation, and TBA to create a red adduct. A 200 µl sample was combined with freshly prepared 20% trichloroacetic acid (TCA), placed in an ice bath for 15 minutes, and then centrifuged at 2500 rpm for 15 minutes at 0°C. Afterward, 200 µl of the supernatant was separated and mixed with 20% TBA. The solution underwent heating on a water bath for 10 minutes, followed by cooling at room temperature and then in an ice-cold bath for 5 minutes. The absorbance was measured at 532 nm, and the quantity was expressed as nanomoles per gram of tissue weight (Garcia et al., 2005).

Assay of Total Protein

The total protein concentration was determined using the method given by Lowry et al., (1951) (Lowry et al., 1951), employing bovine serum albumin as a reference. Absorbance was measured at 750 nm using spectrophotometry, and the results were expressed as milligrams of protein per milliliter of median nerve.

Histopathology

On day 22, animals were euthanized, and the median nerve was extracted for biochemical analysis and histological examination. The nerve samples were preserved in a 10% formalin fixative solution and subsequently sectioned into 5-µm slices using a microtome. These sections were stained with hematoxylin and eosin and observed under a light microscope to identify structural changes, such as axonal degeneration, hemosiderin deposition, edema, neutrophil infiltration, and nerve cell vacuolization (Ayrancı et al., 2013).

Statistical Analysis

The behavioral results were statistically analyzed using two-way analysis of variance (ANOVA) followed by Bonferroni's post-test in GraphPad Prism Version 8.0 software. For the TBA test and total protein analysis, one-way ANOVA followed by Dunnett's test was employed to assess statistical significance.

Results

Qualitative Analysis of Ethanolic Extract of Acorus calamus

The extract showed the presence of coumarins, volatile oils, alkaloids, and phenolic acid (Table 1) (Figure 2).

Effect of Ethanolic EAC on Randall Selitto test in CCI-Induced Painful Neuropathy

The Randall Selitto apparatus was used to measure the effect of CCI on the paw withdrawal threshold of rats. On day 1, the paw withdrawal threshold was the same for all groups. However, from day 7 onwards, significant improvement was observed in the gabapentin group and EAC group (40 mg/kg) compared to the CCI + vehicle group ($^{***}P < 0.001$) (Figure 3).

TABLE 1: Shows the chemical test and inferences

Sr. No	Test	Inference
1.	**Test for coumarins**	The formation of yellow color confirmed the presence of Coumarins in EAC.
2.	**Test for volatile oil**	The formation of red color confirmed the presence of volatile oil in EAC.
3.	**Mayer's test**	The formation of white creamy precipitate confirmed the presence of alkaloid
4.	**Ferric chloride test**	The formation of Green blue coloration indicated the presence of flavonoids.
5.	**Alkaline reagent test**	Showed negative for flavonoids
6.	**Wagner's test**	The formation of brownish precipitate confirmed the presence of alkaloid
7.	**Litmus test**	Blue litmus turned red whereas no change was observed in red litmus confirming the presence of phenolic acid.

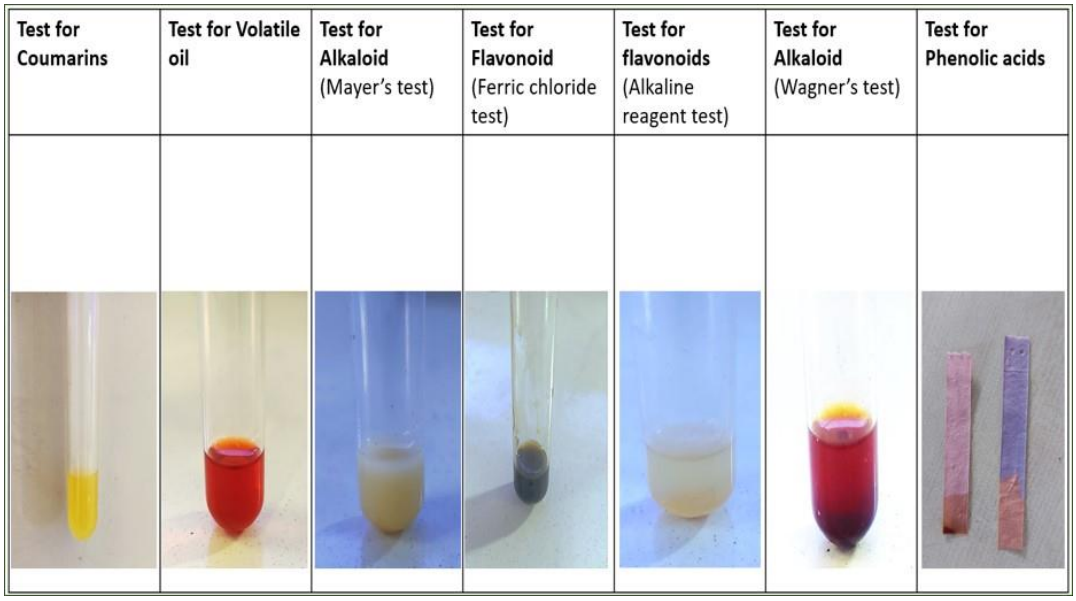

FIGURE 2. Qualitative analysis of ethanolic extract of Acorus calamus.

Effect of ethanolic EAC on Hot Plate Test in CCI-Induced Painful Neuropathy

The Hot Plate test revealed thermal hyperalgesia in the CCI + vehicle group, indicated by reduced jump latency starting from day 7 ($^{***}P<0.001$). Substantial enhancements in jump latency were observed across all treatment groups ($^{***}P<0.001$) from day 7 onwards (Figure 3).

Effect of Chronic Constriction Injury on Median Nerve Functional Index of Wistar Rats

The Walking Track analysis demonstrated a notable decline in the nerve functional index in the CCI + vehicle group from day 1 onwards ($^{***}P<0.001$). Conversely, administration of gabapentin (50 mg/kg) and EAC (20 mg/kg), EAC (40 mg/kg) at both doses notably improved the nerve function index ($^{***}P<0.001$). Comparably, the effect of EAC (40 mg/kg) closely resembled that of gabapentin. However, gabapentin displayed acute effects ($^{*}P<0.05$) not observed in the EAC group (Figure 3).

Effect of CCI of Median Nerve on Body Weight of Wistar Rats

No significant alterations were observed in the total body weight among the groups. Notably, the administration of gabapentin exhibited a significant improvement in body weight compared to the CCI + vehicle group, evident from day 14 onwards ($^{*}P<0.05$ and $^{**}P<0.01$) (Figure 4).

Effect of EAC on CCI of Median Nerve-Induced Lipid Peroxidation in Wistar Rats

Tissue MDA levels were notably elevated ($^{\#\#\#}P<0.001$) in the CCI + vehicle group compared to the normal control group. However, treatment with gabapentin (50 mg/kg), EAC (20 mg/kg), and EAC (40 mg/kg) significant-

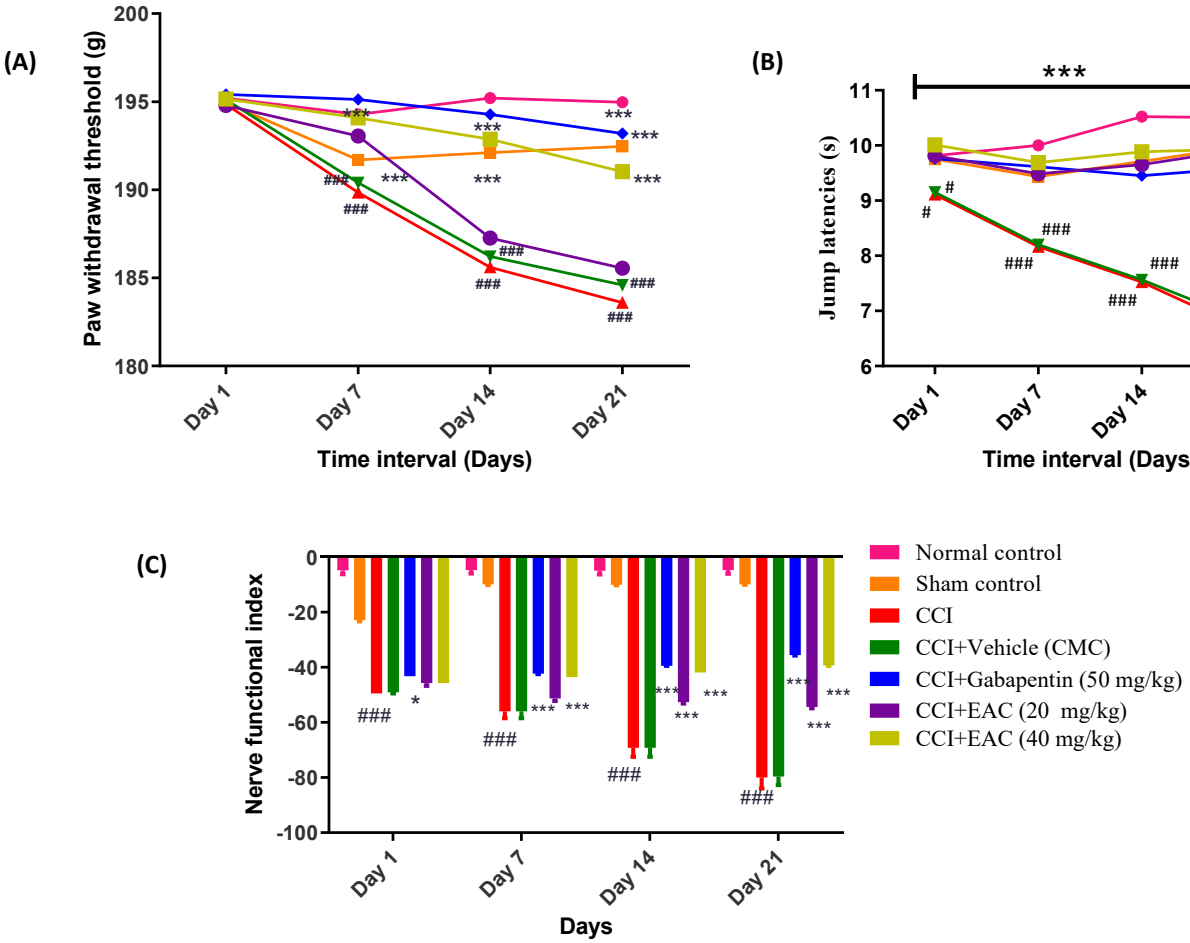

FIGURE 3. Effect of EAC (20 mg/kg), EAC (40 mg/kg), and gabapentin (50 mg/kg) on pressure threshold using A) Randall Selitto apparatus, B) hot plate apparatus, and C) nerve functional index. The data are presented as mean ± SEM (n=8). Statistical significance was determined using two-way ANOVA followed by Bonferroni's test. $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ as compared to the normal control group. $^{\#\#}P<0.01$ as compared to CCI + vehicle group. CCI (Chronic constriction injury), EAC (extract Acorus calamus)

ly mitigated MDA levels ($^{***}P<0.001$) compared to the CCI + vehicle group (Figure 4).

The impact of EAC on alterations in total protein levels induced by CCI of the median nerve in Wistar rats

The animals in the CCI group displayed a significant ($^{\#\#\#}P<0.001$) reduction in total protein compared to the normal control group. Treatment with gabapentin (50 mg/kg), EAC (40 mg/kg), and EAC (20 mg/kg) led to substantial enhancements ($^{***}P<0.001$, $^{*}P<0.05$ respectively) in total protein levels compared to the CCI + vehicle group (Figure 4).

Histopathological Observations

Observations revealed a decrease in the number of Schwann cells and an increase in axonal swelling due to CCI. Treatment with gabapentin and EAC (40 mg/kg) mitigated these pathological changes. However, the protective effects were less pronounced with the lower dose of EAC (Figure 5).

Discussion

CCI, a partial nerve injury model in rats, that is made by tying many ligatures around a nerve and gently restricting it. The animals develop hyperalgesia like behavior as a result of partial nerve injury. The CCI of median nerve in rats correlates to clinical conditions of trauma or tumor developments due to chronic nerve compression in experimental animals which clinically resembles to WMSDs (Dilley et al., 2022). Median nerve neuropathy leads to work-related musculoskeletal disorders, commonly affecting laborers and workers. Females are more susceptible to this disorder. Previous research indicates no discernible difference in tactile al-

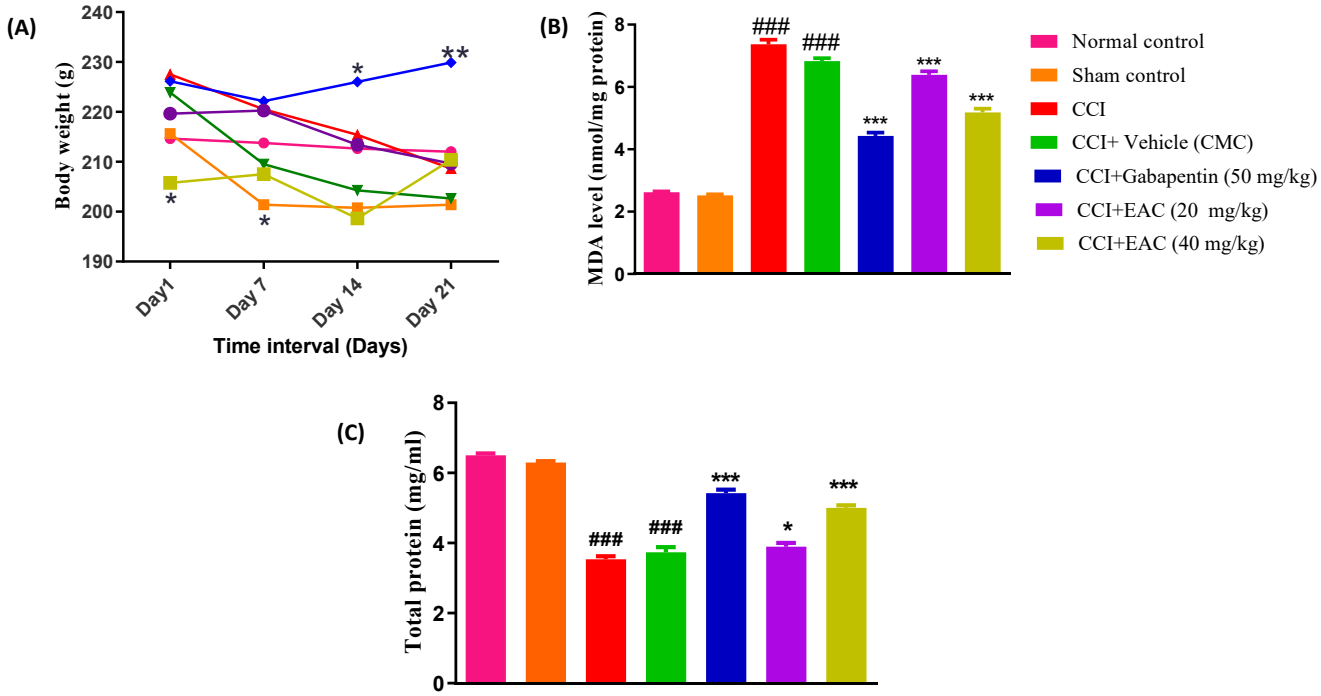

FIGURE 4. Effect of EAC (20mg/kg), EAC (40 mg/kg), and gabapentin (50 mg/kg) on A) body weight, B) MDA levels, and C) total protein levels. The data are presented as mean ± SEM (n=8). Statistical significance was determined using one-way ANOVA followed by Dunnett's post hoc test for MDA level and total protein analysis. Two-way ANOVA followed by Bonferroni's test was used for body weight analysis. $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ as compared to normal control group. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ as compared to CCI + vehicle group. CCI (Chronic constriction injury), EAC (extract Acorus calamus)

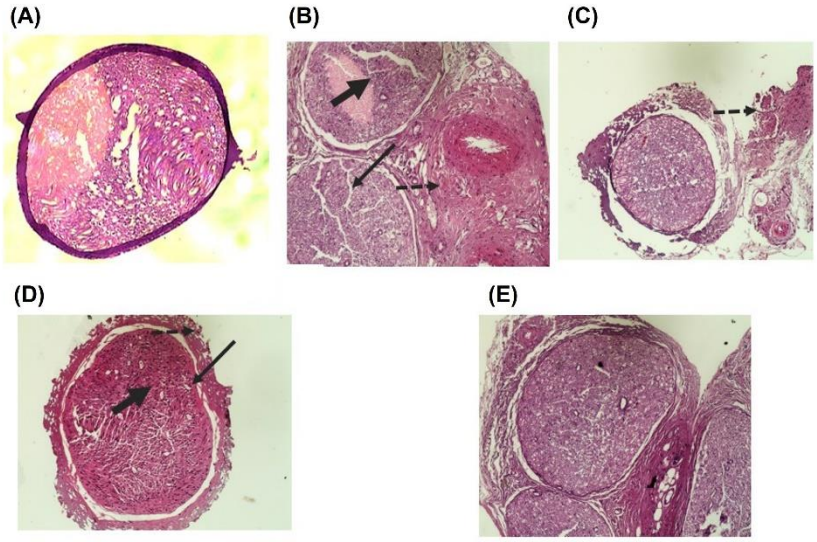

FIGURE 5. Histopathology of nerve cell.
The transverse sections of the median nerve were normalized for the normal, CCI, EAC (20 mg/kg), EAC (40 mg/kg), and gabapentin-treated groups (Figures 5a-e). Figure 5a shows normal fiber arrangement with no observed changes. Figure 5b shows fiber derangement (thin arrow), nerve fiber swelling (bold arrow), and the presence of stimulated Schwann cells (Dotted arrow). Figures 5c-e show the attenuation of CCI-induced neuronal pathological changes and a reduction in myelin sheath derangements seen in EAC (40 mg/kg) and gabapentin (50 mg/kg) treatment groups, respectively (100x) (Figure 5 a, b, c, d, e).

lodynia between male and female rats using nerve ligation methods. This model replicates nerve fiber lesions, mainly affecting the surface of the peripheral nerve—a characteristic feature of carpal tunnel syndrome (Sun-

derland 1976). It has been confirmed that chronic activation of peripheral nociceptors leads to hypersensitivity in primary afferent neurons and central sensitization in dorsal horn neurons (Anwer et al., 2021) (Inquimbert et al., 2018).

In this study, the CCI of the median nerve led to noticeable neuropathic pain, evident through behavioral, biochemical, and histopathological changes. These outcomes align with earlier research conducted by various laboratories. Mechanical nociceptive thresholds were assessed using pressure stimulation, reflecting mechano-hyperalgesia, in accordance with prior methodologies (Randall et al., 1957) and since then have been modified by various other authors.

In this method, the inflamed rat paw is positioned on the apparatus, and pressure is steadily applied to the plantar surface of the paw using a specialized device. Gradually increasing pressure is applied to the right forepaw to measure the nociceptive threshold in grams. Responses such as withdrawal of the forepaw, animal struggle, attempts to bite, or vocalization, such as squeaking, were observed to evaluate the static mechanical nociceptive threshold. This technique is founded on the principle that inflammation enhances sensitivity to pain. The results indicated that treatment of rats with ethanolic EAC as well as gabapentin prolonged the paw withdrawal latencies.

In the hot plate test, as described earlier (Eddy and Leimbach 1953), animals were placed on a preheated platform maintained at 55 °C, and responses such as jumping, paw withdrawal, and paw licking were observed. The parameter assessed in this investigation was the measurement of jump latencies on the hot plate. The results indicated that treatment with EAC prolonged jump latencies compared to the CCI + vehicle group. Similarly, gabapentin also prolonged these latencies. The hot plate method is also used to screen centrally acting analgesics (Witkin et al., 1961), while generally, peripheral analgesics such as aspirin do not affect these responses. Thus, the possibility of a central analgesic effect of EAC extract cannot be dismissed.

The CCI led to a significant increase in median nerve function loss, as evidenced by the elevation in the functional index. This index is a reliable, repeatable, economical, and quantitative measure used to evaluate function subsequent to median nerve injury (Bakare and Owoyele 2020). The animal's paw is labeled with ink and allowed to walk on the platform during this test. The NFI indicates the median functional index of animals evaluated by tracking their footprints on a narrow track. Our findings displayed a significantly enhanced recovery in CCI rats treated with EAC. considering that CCI-induced foot deformities often relate to ongoing pain (Nakazato-Imasato and Kurebayashi 2009), our results suggest that the improved median nerve functional index in EAC-treated CCI rats might be linked to the reduction of spontaneous ongoing pain and the regeneration of the injured median nerve and its axons.

Certainly, carpal tunnel syndrome (CTS) brings considerable discomfort due to median nerve compression. Typically, individuals diagnosed with CTS are often advised to manage their weight effectively as part of pain management and relief strategies (Marchand et al., 2005). Gabapentin is often the preferred medication for alleviating CTS discomfort. However, prolonged use of this drug can lead to weight gain, cognitive disorientation, and gastric irritation, complicating the treatment process. Previous reports have highlighted the association between chronic gabapentin use and increased body weight, which was also observed in our recent findings. Interestingly, our experimental drug EAC showed no impact on body weight gain. Yet, further studies on EAC are necessary to contribute to its safety profile.

Additionally, the release of pro-inflammatory mediators from local macrophages and Schwann cells marks one of the initial stages in the inflammatory response triggered by nerve injuries.

(Woolf and Mannion 1999). Subsequently, there's an infiltration of inflammatory cells, including macrophages, from nearby blood capillaries to the region surrounding the nerve injury. Studies indicate that chronic activation of peripheral nociceptors induces hypersensitivity in primary afferent neurons and leads to central sensitization in dorsal horn neurons (Naik et al., 2006).

Experimental evidence indicates that animals subjected to CCI show heightened MDA levels in nerve tissue. This elevation is attributed to increased oxidative stress, directly correlating with the severity of tissue injury and serving as an index of lipid peroxidation in biological tissues (Hilaire et al., 2005). There were changes in total protein activity (Shukla et al., 2006).

We corroborate similar findings in median nerve tissue, noting an increase in oxidative stress manifested by reduced total protein levels and elevated MDA lev-

els, indicative of lipid peroxidation. Remarkably, EAC treatment significantly mitigated these effects. This supports the claim that EAC effectively attenuated CCI-induced oxidative stress, leading to the amelioration of neuropathic pain. Our findings, coupled with existing literature, strongly indicate the therapeutic potential of EAC in managing neuropathic pain. The main phytoconstituent, β-asarone, is implicated in these beneficial effects, attributed to its anti-oxidative and neuroprotective actions. Furthermore, EAC has demonstrated potential anti-inflammatory effects in prior studies (Muthuraman and Singh 2011; Xavier et al., 2012). Schwann cells, found in the peripheral nervous system (PNS), are pivotal for peripheral nerve healing and regeneration. After nerve damage, these cells display rapid proliferation and migration, actively participating in the repair process. They, along with macrophages, envelop the degenerating myelin sheath, fostering axonal regrowth. Additionally, Schwann cells secrete an array of neurotrophic factors, adhesion molecules, and extracellular matrix components, all crucial for encouraging axonal elongation, myelin formation, and reinnervation (Wang et al., 2015). The histopathological examination in our current study revealed a notable increase in the number of Schwann cells at the injury site, suggesting active neuronal repair. This observation might be attributed to the anti-inflammatory and antioxidant properties previously associated with EAC (Devi and Ganjewala 2011).

Conclusion

In conclusion, our study demonstrated that EAC effectively mitigated the behavioral, biochemical, and histological alterations induced by CCI in rats. These findings suggest the potential of EAC in treating CCI-induced painful peripheral neuropathy and related conditions such as WMSDs, commonly associated with nerve compression in the upper extremities. The observed anti-oxidative, anti-inflammatory, and neuroprotective actions of EAC likely contribute to its therapeutic effects in CCI-induced painful peripheral neuropathy in rats. However, further investigations are warranted to isolate the responsible phytoconstituents and elucidate their mechanisms of action.

Conflict Of Interest

No potential conflict of interest relevant to this article was reported.

Acknowledgements

The authors would like to acknowledge Dr. A. P. Pawar, Principal In-charge, Poona College of Pharmacy, Erandwane, Pune, India, for infrastructural facilities.

Funding

No funding to declare.

Ethics approval

Institutional Animal Ethics Committee (IAEC) of the institute approved experimental protocol. The CPCSEA registration. no of the institute is 1703/PO/Re/S/01/CPCSEA and protocol approval number is by IAEC of institute is IAEC/PCP/PCL35 /2020-2021.

References

Anwer S, Li H, Antwi-Afari MF, Wong A. Associations between physical or psychosocial risk factors and work-related musculoskeletal disorders in construction workers based on literature in the last 20 years: A systematic review. Int J Ind Ergon 2021; 83: 103113. https://doi.org/10.1016/j.ergon.2021.103113

Ayrancı E, Altunkaynak BZ, Aktaş A, Rağbetli M, Kaplan S. Prenatal exposure of diclofenac sodium affects morphology but not axon number of the median nerve of rats. Folia Neuropathol 2013; 51: 76-86. https://doi.org/10.5114/fn.2013.34199

Bain JR, Mackinnon SE, Hunter DA. Functional evaluation of complete sciatic, peroneal, and posterior tibial nerve lesions in the rat. Plast Reconstr Surg 1989; 83: 129-38. https://doi.org/10.1097/00006534-198901000-00024

Bakare AO, Owoyele BV. Antinociceptive and neuroprotective effects of bromelain in chronic constriction injury-induced neuropathic pain in Wistar rats. Korean J Pain 2020; 33: 13-22. https://doi.org/10.3344/kjp.2020.33.1.13

Baron R, Freynhagen R, Tölle TR, Cloutier C, Leon T, Murphy KT, et al. The efficacy and safety of pregabalin in the treatment of neuropathic pain associated with chronic lumbosacral radiculopathy. Pain 2010; 150: 420-427. https://doi.org/10.1016/j.pain.2010.04.013

Bennett GJ, Xie YK. A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. Pain 1988; 33: 87-107. https://doi.org/10.1016/0304-3959(88)90209-6

Bertelli JA, Taleb M, Mira JC, Calixto JB. Muscle fiber type

reorganization and behavioral functional recovery of rat median nerve repair with vascularized or conventional nerve grafts. Restor Neurol Neurosci 1996; 10: 5-12. https://doi.org/10.3233/RNN-1996-10102

Chen JJ, Lue JH, Lin LH, Huang CT, Chiang R PY, Chen CL, et al. Effects of pre-emptive drug treatment on astrocyte activation in the cuneate nucleus following rat median nerve injury. Pain 2010; 148: 158-66. https://doi.org/10.1016/j.pain.2009.11.004

Devi SA, Ganjewala D. Antioxidant Activities of Methanolic Extracts of Sweet-Flag (Acorus calamus) Leaves and Rhizomes. J Herbs Spices Med Plants 2011; 17: 1-11. https://doi.org/10.1080/10496475.2010.509659

Dilley A, Harris M, Barbe MF, Bove G. Aberrant Neuronal Activity in a Model of Work-Related Upper Limb Pain and Dysfunction. The J of Pain 2022; 23: 852-863. https://doi.org/10.1016/j.jpain.2021.12.004

Eddy NB, Leimbach D. Synthetic analgesics. II. Dithienylbutenyl- and dithienylbutylamines. J Pharmacol Exp Ther 1953; 107: 385-93.

Fujiwara M, Iwata M, Inoue T, Aizawa Y, Yoshito N, Hayashi K, et al. Decreased grip strength, muscle pain, and atrophy occur in rats following long-term exposure to excessive repetitive motion. Neuro Endocrinol. Lett 2017; 7: 1737-49. https://doi.org/10.1002/2211-5463.12315

Garcia YJ, Rodríguez-Malaver AJ, Peñaloza N. Lipid peroxidation measurement by thiobarbituric acid assay in rat cerebellar slices. J Neurosci Methods 2005; 144: 127-35. https://doi.org/10.1016/j.jneumeth.2004.10.018

Hilaire C, Inquimbert P, Al-Jumaily M, Greuet D, Valmier J, Scamps F. Calcium dependence of axotomized sensory neurons excitability. Neurosci Lett 2005; 380: 330-4. https://doi.org/10.1016/j.neulet.2005.01.068

Inquimbert P, Moll M, Latremoliere A, Tong CK, Whang J, Sheehan GF, et al. NMDA Receptor Activation Underlies the Loss of Spinal Dorsal Horn Neurons and the Transition to Persistent Pain after Peripheral Nerve Injury. Cell Rep 2018; 23: 2678-89. https://doi.org/10.1016/j.celrep.2018.04.107

Krishnan KS, Raju G, Shawkataly O. Prevalence of work-related musculoskeletal disorders: Psychological and physical risk factors. *Int J Environ Res Public Health* 2021; 18: 9361. https://doi.org/10.3390/ijerph18179361

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951; 193: 265-75.

Marchand F, Perretti M, McMahon SB. Role of the immune system in chronic pain. Nat Rev Neurosci 2005; 6: 521-32. https://doi.org/10.1038/nrn1700

Muthuraman A, Singh N. Attenuating effect of Acorus calamus extract in chronic constriction injury induced neuropathic pain in rats: an evidence of anti-oxidative, anti-inflammatory, neuroprotective and calcium inhibitory effects. BMC Complement Altern Med 2011; 11-24. https://doi.org/10.1186/1472-6882-11-24

Muthuraman A, 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. J Ethnopharmacol 2012; 142: 723-31. https://doi.org/10.1016/j.jep.2012.05.049

Naik A K, Tandan SK, Kumar D, Dudhgaonkar SP. Nitric oxide and its modulators in chronic constriction injury-induced neuropathic pain in rats. Eur J Pharmacol 2006; 530: 59-69. https://doi.org/10.1016/j.ejphar.2005.11.029

Nakazato-Imasato E, Kurebayashi Y. Pharmacological characteristics of the hind paw weight bearing difference induced by chronic constriction injury of the sciatic nerve in rats. Life Sci 2009; 84: 622-6. https://doi.org/10.1016/j.lfs.2009.02.014

Ortega-Álvaro A, Berrocoso E, Rey-Brea R, Leza JC, Mico JA. Comparison of the antinociceptive effects of ibuprofen arginate and ibuprofen in rat models of inflammatory and neuropathic pain. Life Sci. 2012; 90: 13-20. https://doi.org/10.1016/j.lfs.2011.10.002

Rajput SB, Tonge MB, Karuppayil SM. An overview on traditional uses and pharmacological profile of acorus calamus Linn. (Sweet flag) and other acorus species. Phytomedicine 2014; 21: 268-276. https://doi.org/10.1016/j.phymed.2013.09.020

Randall LO, Selitto JJ, Valdes J. Anti-inflammatory effects of xylopropamine. Arch Int Pharmacodyn Ther 1957; 113: 233-49.

Shukla PK, Khanna VK, Ali M, Maurya R, Handa S, Srimal RJPr. Protective effect of Acorus calamus against acrylamide induced neurotoxicity. Phytother Res 2002; 16: 256-60. https://doi.org/10.1002/ptr.854

Shukla PK, Khanna VK, Ali MM, Maurya R, Khan MY, Srimal RC. Neuroprotective effect of Acorus calamus against middle cerebral artery occlusion-induced ischaemia in rat. Hum Exp Toxicol 2006; 25: 187-94. https://doi.org/10.1191/0960327106ht613oa

Silverstein B, Clark R. Interventions to reduce work-related musculoskeletal disorders. J Electromyogr. Kinesiol 2004;14:135-52. https://doi.org/10.1016/j.jele-

kin.2003.09.023

Sunderland S. The nerve lesion in the carpal tunnel syndrome. J Neurol Neurosurg Psychiatry 1976; 39: 615-26. http://dx.doi.org/10.1136/jnnp.39.7.615

Wang L, Sanford MT, Xin Z, Lin G, Lue TF. Role of Schwann cells in the regeneration of penile and peripheral nerves. Asian J Androl 2015; 17: 776-82. http://dx.doi.org/10.4103/1008-682X.154306

Witkin LB, Heubner CF, Galdi F, O'Keefe E, Spitaletta P, Plummer AJ. Pharmacology of 2-amino-indane hydrochloride (Su-8629): a potent non-narcotic analgesic. J Pharmacol Exp Ther 1961; 133: 400-8.

Woolf CJ, Mannion RJ. Neuropathic pain: aetiology, symptoms, mechanisms, and management. Lancet 1999; 353: 1959-64. https://doi.org/10.1016/S0140-6736(99)01307-0

Xavier AM, Serafim KG, Higashi DT, Vanat N, Flaiban KK, Siqueira CP, et al. Simvastatin improves morphological and functional recovery of sciatic nerve injury in Wistar rats. Injury 2012; 43: 284-9. https://doi.org/10.1016/j.injury.2011.05.036

Yousuf S, Marifatul Haq S, Rasool A, Zulfajri M, Hanafiah MM, Nafees H, et al. Evaluation of antidepressant activity of methanolic and hydroalcoholic extracts of Acorus calamus L. rhizome through tail suspension test and forced swimming test of mice. Journal of Traditional Chinese Medical Sciences 2020; 7: 301-07. https://doi.org/10.1016/j.jtcms.2020.07.002

Zulazmi NA, Gopalsamy B, Farouk AA, Sulaiman MR, Bharatham BH, Perimal EK. Antiallodynic and antihyperalgesic effects of zerumbone on a mouse model of chronic constriction injury-induced neuropathic pain. Fitoterapia 2015; 105: 215-21. https://doi.org/10.1016/j.fitote.2015.07.011