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Neuroprotective effect of saponin rich extract of *Acorus calamus* L. in rat model of chronic constriction injury (CCI) of sciatic nerve-induced neuropathic pain

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ABSTRACT

Ethnopharmacological relevance: Traditionally, *Acorus calamus* has been used for the treatment and management of headache, migraine, body ache and severe inflammatory pain in the Unani, Ayurveda and Indian system of medicine.

Aim of the study: Present study focuses on the evaluation of saponin rich extract of *Acorus calamus* (SRE-*AC*) in chronic constriction injury (CCI) of sciatic nerve induced neuropathic pain and neuronal functional changes in rats.

Materials and methods: The pain sensitive tests, i.e., thermal and mechanical hyperalgesia and sciatic functional index test, were performed on the different days, i.e., days 0, 1, 7, 14, and 21. The motor and sensory nerve conduction velocity was also measured on the 21st day. Tissue total protein, superoxide anion generation, total calcium, myeloperoxidase and TNF-α levels were estimated to assess biochemical changes. Histopathological evaluations were also performed. SRE-*AC* (20 and 40 mg/kg) and pregabalin (10 mg/kg, serving as a positive control) were administered orally for 14 consecutive days from the day of surgery.

Results: CCI produced significant ($P < 0.05$) increase in thermal and mechanical hyperalgesia, rise in sciatic functional index, decrease in nerve conduction velocity, along with biochemical and histopathological changes. Oral administration of SRE-*AC* and pregabalin significantly ($P < 0.05$) ameliorated CCI-induced nociceptive pain threshold, sciatic functional and electrophysiological changes in a dose dependent manner. Further, tissue biochemical and histopathological changes were also attenuated.

Conclusion: SRE-*AC* has shown ameliorative effect in CCI-induced neuropathic pain which may be attributed to its multiple actions including anti-oxidative, anti-inflammatory and neuroprotective actions.

1. Introduction

The International Association for the Study of Pain (IASP) defines neuropathic pain as "pain initiated or caused by a primary lesion or dysfunction or transitory perturbation in the peripheral or central nervous system" (Woolf and Mannion, 1999; O'Connor and Dworkin, 2009). It is characterized by the sensory abnormalities such as unpleasant abnormal sensation (dysesthesia), an increased response to painful stimuli (hyperalgesia), and pain in response to a stimulus that does not normally provoke pain (allodynia) (Burakgazi et al., 2011). Peripheral neuropathic pain is observed frequently in patients with long standing diabetes, cancer, AIDS, leprosy, cervical disc protrusion, foraminotomy, and post-surgical event (Muthuraman and Singh, 2011).

Conventional therapy for neuropathic pain such as non-steroidal anti-inflammatory drugs, opioids, tricyclic anti-depressants, anti-convulsants and topical medicine is associated with various adverse effects, withdrawal syndromes and multiple pathological mechanisms and is not suitable for all types of neuropathy (Wiffen et al., 2010). Recently, some preclinical outcome have shown therapeutic efficacy of drugs from plant origin such as *Aconiti tuber*, *Acorus calamus*, *Cannabis sativa*, *Nigella sativa*, *Ocimum sanctum* and *Ginkgo biloba* in neuropathic pain (Comelli et al., 2008; Muthuraman et al., 2008a; Kim et al., 2009; Muthuraman and Singh, 2011). This is also supported by few clinical studies which have recently evidenced the beneficial effect of herbal medicines in neuropathic pain syndrome (Ellis et al., 2009; Chen et al., 2011).

Acorus calamus has a very long history of medicinal use in many herbal traditions. For centuries, many native American tribes were familiar with calamus and it had been used as folk medicine. As per Indian Ayurveda it has high value as a

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rejuvenator for the brain and nervous system and also used for digestive disorders, toothache and headaches (Chevallier, 1996). In Indian and Chinese traditional medicines, roots and rhizomes of *Acorus calamus* have been used for the improvement of age-dependent learning performances, and as carminative, expectorant, anti-fungal, hallucinogenic, hypotensive and sedative (Ghosh, 2006; Hazra et al., 2007). Traditionally, *Acorus calamus* has been used for the treatment and management of headache, migraine, body ache and severe inflammatory pain in the Unani, Ayurveda and in Indian system of medicine. The rhizome part of *Acorus calamus* has been commonly used to relieve the muscle, joint, vascular and nerve injury associated severe inflammatory and neuropathic pain in south Indian population (Muthuraman and Singh, 2011). *Acorus calamus* has various phytochemical constituents such as saponins, glycosides, flavonoids, tannins, polyphenolic compounds, mucilage, volatile oil and bitter principles (Mittal et al., 2009; Raja et al., 2009).

Saponins are glycosylated plant secondary metabolites found in food and many major medicinal plants. Saponins play a numerous functional and pathophysiological role in the biological systems such as immune responses (Zhai et al., 2011), phagocytic action (Kang et al., 2008), anti-convulsant (Jalsrai et al., 2010), neuroprotective and neuropathic pain (Kaur et al., 2010). Triterpenoid saponins of *Ocimum sanctum* have been reported to produce the anti-neuralgic effect (Kaur et al., 2010). *Acorus calamus* is also known to possess the triterpenoid saponins (Raja et al., 2009). The saponin constituent of *Acorus calamus* has been reported to possess the neuropharmacological activities (Parap and Mengi, 2003; Jayaraman et al., 2010). Our previous studies have documented that hydroalcoholic extract of *Acorus calamus* produces beneficial effect in various rat models of neuropathic pain (Muthuraman and Singh, 2011; Muthuraman et al., 2011a, 2011b). However, saponin rich fraction of *Acorus calamus* remains to be evaluated for its potential in neuropathic pain. Therefore, the present study has been designed to investigate the efficacy of saponin rich extract of *Acorus calamus* in chronic constriction injury of sciatic nerve-induced neuropathic pain in rats.

2. Materials and methods

2.1. Drugs and chemicals

Pregabalin (gift sample obtained from Ranbaxy Research Laboratories, Gurgaon), Ehrlich reagent (SRL, Mumbai), nitroblue tetrazolium (NBT, Sigma Aldrich, USA), hexadecyl trimethyl ammonium bromide (HETAB), *O*-dianisidine hydrochloride (S.D. Fine, Mumbai, India), Folin–Ciocalteu's phenol reagent (Merck Limited, Mumbai), 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB), bovine serum albumin (BSA, Sisco Research Laboratories Pvt. Ltd., Mumbai) were procured for the present study. All the reagents used in the present study were of analytical grade.

2.2. Plant material

The fresh rhizome part of *Acorus calamus* was collected at Kodaikanal, Tamilnadu, India and was authenticated through Department of Botany, American College, Madurai District, Tamilnadu. Plant sample has been kept in voucher specimen (PUP-218/2009–2010) at Punjabi University, Patiala for future reference. After authentication, fresh rhizome of *AC* was collected, cleaned thoroughly with distilled water and dried under shade. The shade dried rhizome was pulverized in a mechanical grinder to obtain coarse powder (sieve no.10/40).

2.3. Preparation of hydroalcoholic extract of Acorus calamus (HAE-AC)

Hydroalcoholic extract was prepared from coarse rhizome powder of *Acorus calamus* as described in the method of Parap and Mengi (2003) and Muthuraman et al. (2011a, 2011b). The coarsely powdered plant material was subjected to extraction with mixture of ethanol:water (1:1, 50%) at room temperature followed by vacuum drying at low temperature ($<50\,^{\circ}C$). The yield of hydroalcoholic extract was found to be 26.4 w/w%.

2.4. Preparation of saponin rich extract of Acorus calamus from HAE-AC (SRE-AC)

Saponins rich extract was prepared from aqueous extract of *Acorus calamus* (SRE-*AC*) as described in the method of Harborne (1988) with some modification. Briefly, the aqueous extract of *Acorus calamus* (100 g) was refluxed with *n*-butanol for 2 h and *n*-butanol soluble constituents were separated by filtration. The *n*-butanol layer was sequentially washed with distilled water, alkali (2% KOH) and distilled water again. The *n*-butanol layer was evaporated and dried under vacuum to obtain a clear powder of crude saponins. The purity of saponin in SRE-*AC* was analyzed by high performance liquid chromatography with diode-array detection (HPLC-DAD) technique, using either reverse phase column. Diosgenin was used as an (external standard) HPLC chromatogram of marker compound for saponin analysis. The chromatographic analysis was performed on a C18 column (4.6×150 mm). The experimental conditions were an isocratic binary system of acetonitrile/water (90:10), a flow rate of 1 ml/min and a temperature of 35 °C. The injection volume was 20 μl and detection was performed at 194 nm, according to the procedure described by Oncina et al. (2000). The retention times and spectra were compared with that of authentic standards.

2.5. Determination of total saponins

The total saponins content of SRE-*AC* was determined by the vanillin–sulfuric acid method as described by Hiai et al. (1976). This extract was mixed with vanillin (8 w/v%) and sulfuric acid (72 w/v%). The mixture was incubated at 60 °C for 10 min, cooled in an ice water bath for another 15 min followed by the absorbance measurement at 538 nm. Ursolic acid was used as a reference standard and the content of total saponins was expressed as ursolic acid equivalents (UA mg/mg extract).

2.6. Experimental animals

Wistar rats of either sex, weighing 200–230 g (procured from Punjab Agriculture University, Ludhiana), were employed in the present study. They were housed in the animal cages with free access to water and standard laboratory pellet chow diet. The rats were exposed to 12 h light and dark cycles. The experimental protocol was duly approved by the Institutional Animal Ethics Committee and the care of the animals was carried out as stated in the guidelines of the Committee for the Purpose of Control and supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, Government of India (Reg. No. 107/1999/CPCSEA).

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

Peripheral neuropathy was induced in rats by chronic constriction injury as described in the method of Bennett and Xie (1988) with slight modifications in Sommer and Schafers (1998).