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Ameliorative effects of *Ocimum sanctum* in sciatic nerve transection-induced neuropathy in rats

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ABSTRACT

Objectives: The present study was aimed at investigating the ameliorative effect of *Ocimum sanctum* in sciatic nerve transection (axotomy)-induced peripheral neuropathy in rats.
Materials and methods: Sciatic nerve transection-induced axonal degeneration was assessed histopathologically. Paw pressure, Von Frey Hair, tail cold-hyperalgesia, motor in-coordination tests were performed to assess the extent of neuropathy. Biochemical estimations of thiobarbituric acid reactive species (TBARS), reduced glutathione (GSH), and total calcium levels were also performed. Methanolic extract of *Ocimum sanctum* at different doses (50, 100 and 200 mg/kg *p.o.*) was administered for 10 consecutive days starting from the day of surgery.
Results: Administration of *Ocimum sanctum* attenuated sciatic nerve transection-induced axonal degeneration, reduction of nociceptive threshold and motor in-coordination. Moreover, it also attenuated axotomy-induced rise in TBARS, total calcium and decrease in GSH levels in a dose-dependent manner.
Conclusion: Anti-oxidant and calcium attenuating actions may be responsible for observed ameliorative effects of *Ocimum sanctum* in axotomy-induced neuropathy.

1. Introduction

Neuropathic pain associated with peripheral nerve injury is characterized by 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) (Woolf and Mannion, 1999). Axotomy, complete transection of peripheral nerve, is widely employed as an experimental model for inducing peripheral neuropathy in rats. Axotomy-induced neuropathy in experimental animals refers to Complex Regional Pain Syndrome (CRPS) in humans (Pramod, 2006), which is very common following fracture, total knee arthroplasty and stroke (Daviet et al., 2002). Though some drugs have been found to be effective in managing the symptoms of neuropathy, yet their full clinical exploitation is limited due to wide spectrum of adverse effects associated with their clinical use. Moreover, none of the medications assessed in randomized controlled studies conducted has been found effective in CRPS (Kalita et al., 2006). So, there has been an urgent need of alternative medicine for managing neuropathy particularly in CRPS.

Ocimum sanctum (L.), (syn, Tulsi) is an indigenous plant commonly found in India and is recommended in the ayurveda for the treatment of bronchitis, bronchial asthma, malaria, diarrhea, dysentery, skin diseases, arthritis, painful eye diseases, chronic fever and insect bite. Experimental reports have indicated its protective effects against genotoxicants, chemical carcinogens, ischaemic cerebral injury, ischaemia-reperfusion and isoproterenol-induced myocardial damage. Moreover, its hepato-protective, immuno-modulatory, anti-ulcer, anti-diabetic, anti-hypercholesterolaemic, chemo-protective, nootropic, anti-tussive, anti-inflammatory, wound healing, anti-tumorigenesis, anti-convulsant, anthelmintic, anti-bacterial, anti-giardial and anti-stress activities have also been documented (Jaggi et al., 2003; Yanpallewar et al., 2004; Mohanty et al., 2006; Niture et al., 2006; Anu et al., 2007; de Almeida et al., 2007; Jagetia, 2007; Jyoti et al., 2007). *Ocimum sanctum* leaves contain various bioactive constituents such as flavanoids like luteolin, orientin, vicenin; triterpenoids like ursolic acid; fixed oils like palmitic, stearic, oleic, linoleic, linolenic; essential oil like eugenol, camphor, carvacrol, caryophyllene, decylaldehyde, nerol, α-pinene, γ-selinene, cirsilineol, cirsimartin, isothymusin, isothymosin, tannins, etc. (Kelm et al., 2000).

Fresh leaf extract/decoction of *Ocimum sanctum* with hot water is very commonly used to relieve muscular pain, joint pain and severe headache in south India, particularly at Thoothukkudy, Mudivaithanendal, Thirunelvelly and Kannyakumari region.

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Moreover, experimental reports also suggest its potent analgesic activity (Godhwani et al., 1987; Khanna and Bhatia, 2003). Traditionally, it has been used as nerve tonic to alleviate the problems related to nerves. However, its potential role in neuropathic pain is still unexplored. So, the present study was designed to investigate the possible ameliorative effect of *Ocimum sanctum* in sciatic nerve transection-induced painful neuropathy in rats.

2. Materials and methods

2.1. Plant material

Fresh leaves of *Ocimum sanctum* were collected from Ooty and authenticated through Government Arts College, Ooty. Plant sample has been kept in Voucher specimen (pup-025/2006–2007) at Punjabi university, Patiala.

2.2. Extraction

The fresh leaves of *Ocimum sanctum* were shed dried at room temperature and reduced to coarse powder. The powder was extracted with mixture of methanol:water (3:1). The solvent was completely removed at 50 °C under reduced pressure. The yield of the extract was 13% (w/w) in terms of dried starting material. The extract was found to contain polyphenols, glycosides and saponins.

2.3. Chemicals

DTNB (5,5′-dithio, bis(2-nitrobenzoic acid), BSA (bovine serum albumin), reduced glutathione were purchased from Sisco Research Laboratories, Mumbai. Thiobarbituric acid was purchased from Loba Chemie, Mumbai. All other reagents were obtained from S.D. Fine chemicals, Mumbai, India.

2.4. Animals

Wistar–albino rats of either sex weighing 180–250 g, maintained on standard laboratory diet (Kisan Feeds Ltd., Mumbai, India) and having free access to tap water, were employed in the present study. They were housed in the departmental animal house and were exposed to 12 h cycle of light and dark. The experimental protocol was approved by Institutional Animal Ethics Committee and care of the animals was carried out as per the guidelines of 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).

2.5. Induction of peripheral neuropathy by axotomy

Peripheral neuropathy was induced in rats by complete sciatic nerve transection (axotomy) as described by Wall et al. (1979). Briefly, rats were anesthetized with thiopental sodium (35 mg/kg *i.p.*). The skin of the lateral surface of the left thigh was incised and a cut was made directly through the biceps femoris muscle to expose the sciatic nerve. It was tightly ligated with silk at two locations before trifurcation of the terminal branches (the sural, common peroneal and tibial nerves). The sciatic nerve was transected between the ligatures, approximately 5 mm of length, in the left paw. No surgery was performed on the right thigh and uninjured right paw served as control. Nociceptive thresholds were assessed before and after performing surgery on different day intervals up to 14th day.

3. Behavioral studies and pharmacological studies

3.1. Paw pressure test

Mechanical nociceptive threshold, an index of mechano-hyperalgesia, was assessed by method described by Randall and Selitto (1957). Briefly, nociceptive threshold, expressed in grams, as measured by applying increasing pressure to the left hind paw. Withdrawal of hind paw was used to assess the nociceptive threshold. The cut-off pressure was 450 g.

3.2. Von Frey Hair test

Mechano-tactile allodynia (non-noxious mechanical stimuli) was assessed as described by Chaplan et al. (1994). Briefly, calibrated nylon filaments, in terms of different bending forces, were applied to the mid-plantar surface of left hind paw. The filaments were applied 10 times, starting with the softest and continuing in ascending order of stiffness. A brisk withdrawal of the hind limb was considered a positive response. The criterion for the threshold value, in grams, was equal to the filament evoking a withdrawal of the paw 5 times out of 10 trials, i.e., 50% response.

3.3. Tail immersion test (tail cold-hyperalgesia test)

Spinal thermal sensitivity was assessed by the tail immersion test as described by Necker and Hellon (1978). Briefly, the terminal part of the tail (1 cm) of the rat was immersed in cold-noxious temperature (0–4 °C), until the tail was withdrawn. The duration of the tail withdrawal reflex was recorded and a cut-off time of 15 s was used.

3.4. Motor coordination test

Motor coordination was evaluated by a Rota-Rod device as described by Jones and Roberts (1968). Rats were placed for 1 min on the rotating rod. The time taken for the falling from the roller, during 1 min, was recorded.

4. Biochemical estimation of markers of oxidative stress

After 14 days of surgery, animals were sacrificed by cervical dislocation and sciatic nerve was immediately isolated from the upper part of the axotomised nerve. Tissue homogenate was prepared with 0.1 M Tris–HCl buffer (pH 7.4,) and supernatant of homogenate was employed to estimate total protein content, TBARS, reduced glutathione and total calcium content.

4.1. Estimation of tissue protein

Protein concentration was estimated according to the method of Lowry et al. (1951), using BSA (bovine serum albumin) as a standard.

4.2. Estimation of lipid peroxidation

Estimation of lipid peroxidation was done by measuring the levels of malondialdehyde (MDA) (Okhawa et al., 1979). The concentration of MDA in sciatic nerve homogenates was expressed in terms of nM MDA/mg protein.

4.3. Estimation of reduced glutathione

Reduced glutathione was measured according to the method of Ellman (1959). Equal quantity of sciatic nerve homogenate