WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.045
Volume 3, Issue 6, 955-972. Research Article ISSN 2277 – 7105

AMELIORATIVE EFFECTS OF *MURRAYA KOENIGII* IN CHRONIC CONSTRICTION INJURY OF SCIATIC NERVE INDUCED NEUROPATHIC PAIN IN RATS

Deepika Saini[1], Tapan Behl*[2], Ishneet Kaur[1], Meenu Nagpal[1], Heena Goel[3], Rajesh K. Pandey[4]

[1]Department of Pharmacy, Chandigarh College of Pharmacy, Mohali, Punjab.
[2]Department of Pharmacology, Vallabhbhai Patel Chest Institute, University of Delhi, Delhi.
[3]Department of Animal Husbandry, Junga, Shimla, Himachal Pradesh.
[4]All Excel Inc, 135 Wood St. West Haven CT 06410 USA.

Article Received on
16 June 2014,

Revised on 11 July 2014,
Accepted on 06 August 2014

***Correspondence for Author**
Tapan Behl
Department of Pharmacology, Vallabhbhai Patel Chest Institute, University of Delhi, Delhi.

ABSTRACT

Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage and is induced by noxious stimuli. The sensation of pain is a protective mechanism for the body and it initiates nociceptive reflexes. Neuropathic pain is defined as pain initiated or caused by a primary lesion or dysfunction in the nervous system, either central nervous system (CNS) or peripheral nervous system (PNS). As the onset of neuropathic pain may be delayed after nerve injury, pain may still be present after healing is complete. This makes proper diagnosis and early treatment difficult. Moreover, neuropathic pain commonly occurs as a secondary symptom in diseases like diabetes, cancer and herpes zoster infection; it may also occur with treatments such as chemotherapeutics or cytotoxic drugs. The various first line drugs available for neuropathic pain (such as gabapentin, pregabalin, duloxetime, tricyclic antidepressants etc.,) are not found to be fully effective in the treatment. Henceforth, alternative treatments are being researched upon. *Murraya koenigii* is one such possible alternative, which is considered in the present study and is successfully found to provide positive results towards the prevention and maintenance of peripheral neuropathic pain due to its anti-oxidant, anti-inflammatory and cellular calcium modulatory action.

KEY WORDS: - Thermal hyperalgesia, cold allodynia, reduced glutathione (GSH), pregabalin, neuroprotective

INTRODUCTION

Neuropathic pain is a neurodegenerative disorder, which consists of a wide range of heterogeneous chronic conditions that are characterized by the presence of abnormal and unpleasant sensory symptoms, such as allodynia (pain in response to a stimulus which does not normally provoke pain) and hyperalgesia (an increased response to a normally painful stimulus) [1]. According to the most recent classification, The International association for the Study of Pain (IASP) defines neuropathic pain as "pain initiated or caused by a lesion or dysfunction in the somatosenory nervous system either the peripheral nervous system (PNS) or central nervous system (CNS)" [1-3]. The exact prevalence of neuropathic pain is not known. Population-based surveys designated to investigate chronic pain with neuropathic characteristics have reported the prevalence of neuropathic pain to be 7-8% in the general population [4-6]. The severity of chronic pain varies from person to person, between old and young and even sex. Sometimes, lesions similar in appearance may cause pain in one person but no pain in another [7]. Neuropathic pain has been described problematic because it is often in parts of body that appear normal. It is generally chronic, severe and resistant to over the counter analgesics [8]. Various pathological and clinical conditions such as long standing diabetes, cancer, AIDS-related neuropathy, leprosy, post herpetic neuralgia, cervical disc protrusion, post amputation stump (phantom limb pain), trigeminal neuralgia and multiple sclerosis have been noted to trigger neuropathic pain [9].

There is a large availability of clinically relevant animal models to study neuropathic pain which are made by producing diseases or causing injuries to the spinal cord or peripheral nerves. In the present study, model of chronic constriction injury of sciatic nerve was used. Chronic constriction injury (CCI) of sciatic nerve induced painful neuropathy is a widely employed animal model of neuropathic pain proposed by Bennett and Xie in 1988, for induction of neuropathic pain in experimental animals. It shares the pathophysiology of carpel tunnel syndrome and tarsal tunnel syndrome in humans due to the entrapment of median nerve in narrowing carpel tunnel. CCI induced neuropathy in experimental animals also mimics Complex Regional pain Syndrome dystrophy (CRPS-RSD) in humans [10-12].

The current treatments such as the use of tricyclic anti-depressants (amitriptyline and duloxetine), anti-convulsants (gapapentin, pregabalin and carbamazepine) and topical treatments (lidocaine patch, capsaicin) are proved to be ineffective and produce severe side effects [8, 13-14]. Among opioids, the widely used drug for the treatment of chronic to moderate pain is morphine. But its therapeutic value is still controversial because in some patients with

neuropathic pain, opioids are reported to be ineffective and produce multiple side effects, particularly severe CNS toxicity [15-16]. After decades of serious obsession with the modern medicinal system, people have started looking at the ancient healing systems like Ayurveda, Siddha and Unnani. This is because of the adverse effects associated with the synthetic drugs. Herbal drugs play an important role in health care programs especially in developing countries. Some recent clinical reports have also advocated beneficial effect of drugs from plant origin in neuropathic pain conditions [17].

Murraya koenigii, belonging to the family Rutaceae, commonly known as curry-leaf tree, is a native of India, Sri Lanka and other South Asian countries. It is an aromatic shrub found throughout India and mainly cultivated for its aromatic leaves. Leaves are used as a condiment in preparation of curry powder, pickle, chutney, sausages and seasoning [18-19]. The flavor and fragrance of its leaves are retained even after drying [19]. *Murraya koenigii* is widely used in Indian cookery for centuries and have a versatile role to play in traditional medicine. Traditionally, the plant is used as a stimulant, stomachic, febrifuge, analgesic and for the treatment of diarrhea, dysentery and insect bite. It is also used to allay heat of body [20]. The leaves of *Murraya koenigii* are also used in Ayurvedic medicine. It has been found by a study conducted on the effects of this plant that the leaves of this plant have no adverse effects. It was evident from the unchanged blood parameters/constituents and normal histopathology of hepatic tissue in *Murraya koenigii* fed rats. Additionally, it is reported to possess anti-inflammatory, hepatoprotective, antioxidant, neuroprotective, anti-diabetic and hypolipidemic activities [20-24]. These data suggest that *Murraya koenigii* has antinociceptive and neuroprotective activity. However, its role in the development and maintenance of peripheral neuropathic pain is not known.

In an effort to understand the contribution of *Murraya koenigii* in the development and maintenance of neuropathic pain, the present study was designed to investigate the ameliorative effects of *Murraya koenigii* leaf extract in chronic constriction injury induced neuropathic pain inrats. Pregabalin was taken to serve as positive control in this study.

MATERIAL AND METHODS

Experimental Animals

Age matched young Wistar rats of either sex weighing about 200-280g were employed in this study. Animals were housed under standard conditions of light and dark cycle in the Animal House of Chandigarh College of Pharmacy, Landran, Mohali, India with a

balanced rat feed and clean drinking water *ad libitum.* All experiments were carried between 8:00 A.M. to 4:00 P.M. The experimental protocols used in the present study were approved by the Institutional Animal Ethics Committee (IAEC) and were carried out in accordance with the guidelines of Committee for Purpose of Control and Supervision of Experimentation on Animals (CPCSEA), Government of India. All the experiments for different treatment groups were performed using age matched animals to avoid variability between experimental groups. The animals were placed in groups of 5-6 in polypropylene plastic cages for one week till chronic constriction injury of sciatic nerve was done. After surgery, the rats were kept in individual cages at room temperature of 25±2^{o}C during which the animals were gently handled.

Plant Material

The leaves of *Murraya koenigii* were collected, extracted and authenticated by Ayush Herbs and Pharmaceuticals, Kangra, Himachal Pradesh, India.

Drugs and Chemicals

All reagents and chemicals used in this study were of analytical grade and were freshly prepared. Ethanolic extract of *Murraya koenigii* leaves was purchased from Ayush Herbs and Pharmaceuticals (Kangra, HP, India). DTNB (5, 5-dithiobis-2-nitrobenzoic acid), Folin-Ciocalteu phenol reagent, Bovin serum albumin, reduced glutathione (GSH), sulphanilamide, hydrochloric acid, sodium hydroxide, sodium chloride, N-(1-naphthyl) ethylenediamine dihydrochloride, potassium dihydrogen phosphate, were purchased from Loba Chemicals (Mumbai, india). Thiobarbituric acid was purchased from magus Chemicals. Trichloroacetic acid was purchased from Nice Chem. Pvt. Ltd. (Chochin, India). EDTA was purchased from Thomas baker, India. Pregabalin and Ketamine were purchased from Kailash Medicos, Rupnagar, India. Each dose of *Murraya koenigii* leaves extract was freshly prepare in normal saline (0.9% NaCl) and given by oral route.

Induction of chronic constriction injury

The peripheral neuropathy was induced in rats by chronic constriction injury described by method of Bennett and Xie, 1988 [25]. The rats were anesthetized with ketamine and xylazine (80mg/kg and 10 mg/kg, *i.p.* respectively). The hair of the rat's lower back in thigh region of left paw was shaved, and the skin was sterilized with povidine-iodine (Betadine). The skin of the lateral surface of the left thigh was incised and a cut was made directly through the biceps femoris muscles to expose the sciatic nerve. Four ligatures (4-0 chromic gut), were placed

around the nerve proximal part of the trifurcation with a distance of 1 mm between each ligature. The ligatures were loosely tied until a short flick of the ipsilateral hind limb was observed. Great care was taken not to interrupt epineural blood flow during tying the ligatures. After performing nerve ligation, muscular and skin layer were immediately sutured with thread and topical antiseptic was applied at once. In sham-operated rats, the same surgical procedure was followed, the connective tissue was freed, and no ligatures were applied to the sciatic nerve. Nociceptive threshold was assessed before and after performing surgery on different days i.e., 0, 1, 3, 6, 9, 12, 15, 18 and 21st day.

Experimental Groups

Animals were divided in eight groups, each comprising of five Wistar rats.

Group I (Normal control)

Rats were not subjected to any surgical procedure. The behavioral tests were performed on the different days (already stated above). Thereafter, all the animals were sacrificed subjected to biochemical analysis for the estimation of reduced glutathione level lipid peroxidation, total protein and total calcium level.

Group II (Sham control)

Rats were subjected to a surgical procedure (on day 0) to expose the sciatic nerve without any ligation. The behavioral tests were employed before performing the surgery and after 24 hours of surgery (i.e., day 1st) on different days as described. The biochemical analysis was also done as mentioned in group I.

Group III (Chronic Constriction Injury, CCI)

Rats were subjected to a surgical procedure (on day 0) to expose the sciatic nerve. Four loose ligatures were then tied on the nerve. The behavioral tests were employed before performing the surgery and after 24 hours of surgery (i.e., day 1st) on different days as described. The biochemical analysis was also done as mentioned in group I.

Group IV (MK *per se*)

Ethanolic extract of *Murraya koenigii* (400 mg/kg, *p.o.*) was administered for 14 days in normal rats, starting from the day 1st. The behavioral tests and the biochemical parameters were assessed as mentioned in group I.

Group V (Low dose of MK in CCI)

Ethanolic extract of *Murraya koenigii* (100 mg/kg, *p.o.*) was administered for 14 days in rats subjected to CCI, starting from the day 1st. The behavioral tests and the biochemical parameters were assessed as mentioned in group I.

Group VI (Intermediate dose of MK in CCI)

Ethanolic extract of *Murraya koenigii* (200 mg/kg, *p.o.*) was administered for 14 days in rats subjected to CCI, starting from the day 1st. The behavioral tests and the biochemical parameters were assessed as mentioned in group I.

Group VII (High dose of MK in CCI)

Ethanolic extract of *Murraya koenigii* (400 mg/kg, *p.o.*) was administered for 14 days in rats subjected to CCI, starting from the day 1st. The behavioral tests and the biochemical parameters were assessed as mentioned in group I.

Group VIII (Pregabalin in CCI)

Pregabalin (10mg/kg, *p.o.*) was administered for 14 days in rats subjected to CCI, starting from the day 1st. The behavioral tests and the biochemical parameters were assessed as mentioned in group I.

Treatment Schedule

All animals were acclimatized to laboratory environment for at least 2 hours before behavioral testing. All the rats were subjected to behavioral parameters such as: Thermal hyperalgesia and cold allodynia on day 0 before performing surgery and subsequently 2 hours after *Murraya koenigii* leaf extract or pregabalin administration on the specified days. To evaluate the effect of *Murraya koenigii* leaf extract (100, 200 and 400 mg/kg, *p.o.*, once daily) and pregabalin (10 mg/kg, *p.o.*, once daily) (positive control) on the development of neuropathic pain symptoms in rats treatment was started on day 1 i.e., after 24 hours of surgery and continued upto day 14. Sham-operated and nerve injured control animals (CCI control) received equal volume of vehicle before the behavioral testing at the same time when *Murraya koenigii* leaf extract was administered.

Nociceptive threshold was assessed before and after performing surgery on different days, i.e., 0, 1, 3, 6, 9, 12, 15, 18 and 21st day. On day 21, all the animals were sacrificed after

behavioral assays for the biochemical studies i.e., estimation of reduced glutathione level, lipid peroxidation, total protein and total calcium level.

Behavioral Studies

Assessment of Thermal hyperalgesia

Thermal hyperalgesia of the hind paw was assessed by using Eddy's hot plate as described method by Eddy *et al,* 1988 [26], for assessing the reactivity against noxious thermal stimuli. The rats were placed on the top of a controlled preheated (52.5±0.5^{o}C) and maintained hot plate surface. The latency to the first sign of paw licking or jump response of left hind paw was noted as an index of nociceptive pain threshold. The cut-off time of 20 sec was maintained to avoid damage to the paw. Results are expressed as mean time in seconds ± S.E.M.

Assessment of Cold Allodynia

Cold allodynia was assessed after 2 hours of assessment of hyperalgesia by measuring paw withdrawal latency (PWL) of hind paws against non-noxious stimulus when dipped in water bath maintained at 10±0.5^{o}C [27-28]. Baseline latency of paw withdrawal to cold stimulation was measured thrice, 5 minutes apart, and averaged. A cut-off time of 15 seconds was maintained throughout the experimental protocol. Results are expressed as mean time in seconds ± S.E.M. A significant reduction in paw withdrawal latency (PWL) indicated allodynia.

Collection of Blood and Sciatic Nerve in rats

All the animals were sacrificed by cervical dislocation immediately after behavioral assays on day 21st. The ipsilateral sciatic nerve of ach rat and the tissue beneath the sciatic nerve were isolated for the stimulation of markers of oxidative stress and inflammation. The isolated sciatic nerve was weighed and washed with normal saline. A 10% (w/v) sciatic nerve homogenate was prepared in 0.1 M Tris-HCl buffer (Ph 7.4). The tubes with homogenate were kept in ice water for 30 minutes and then centrifuged for 15 minutes at 2000 rpm to obtain the clear supernatant for the estimation of biochemical parameters. Blood samples were collected by retro-orbital puncture just before sacrificing the animal. The blood was kept at room temperature (25^{o}C) for 30 minute after it was centrifuged at 4000 rpm for 15 minutes to separate serum. Serum was used for the estimation of total calcium content.

Biochemical Assessment

Lipid Peroxidation

Concentration of malondialdehyde (MDA), a measure of lipid peroxidation, was assayed in the form of thiobarbituric acid reactive substances (TBARS) as described by the method of Niehaus and Samuelsson, 1968 [29]. In this method, 0.1 ml of supernatant of sciatic nerve homogenate was treated with 2 ml of (1:1:1 ratio) thiobarbituric acid-trichloroacetic acid-hydrochloric acid (TBA-TCA-HCL) reagent. TBARS reagent was prepared by mixing equal volumes of TBA (37%), TCA (15%) and HCl (0.25 N). Then the mixture was placed in hot water bath for 15 minutes, cooled and centrifuged at 1000 rpm for 10 minutes. The absorbance of the clear supernatant was measured at 532 nm (UV-1700 Spectrophotometer, Shimadzu, Japan) against blank. The values were calculated using molar extinction coefficient of chromophore (1.56×10^5 M^{-1} cm^{-1}) and are expressed as nmole per mg of protein.

Estimation of Total Protein

The protein concentration in the sciatic nerve was estimated according to the method described by Lowry *et al.*, 1951 [30] using bovine serum albumin as a standard. 0.15 ml of supernatant of tissue homogenate was diluted to 1 ml with distilled water and then 5 ml of Lowry reagent was added. Lowry reagent was freshly prepared by mixing 1% w/v of copper sulphate, 2% w/v sodium-potassium tartrate, 2% w/v of sodium carbonate in 0.1 ml M NaOH in a ratio of 1:1:98. The contents were mixed thoroughly. The mixture was allowed to stand 15 minutes at room temperature (25^{o}C). Then 0.5 ml of 1:1 v/v diluted Folin-Ciocalteau reagent was added. The content was vortexed vigorously and incubated at room temperature (25^{o}C) for 30 minutes. The protein content was determined spectrophotometrically at 750nm (UV-1700 Spectrophotometer, Shimadzu, Japan) against suitably prepared blank. The amount of total protein was expressed in mg/ml of 10% sciatic nerve homogenate.

Estimation of Reduced Glutathione

The concentration of endogenous antioxidant reduced glutathione (GSH) level in the sciatic nerve was measured according to the method of Ellman, 1959 [31]. Equal quantity of sciatic nerve homogenate was mixed with 10% trichloroacetic acid and centrifuged to separate proteins. To 0.01 mL of this supernatant, 2 ml of phosphate buffer (pH 8.4), 0.5 ml of 5, 5'-dithiobis(2-nitobenzoic acid) and 0.4 ml double-distilled water were added. Mixture was vortexed and the absorbance was taken at 412 nm (UV-1700 Spectrophotometer, Shimadzu,

Japan) against blank within 15 minutes. The concentration of reduced glutathione was expressed as microgm/mg of protein in sciatic nerve tissue.

Estimation of Total Calcium

Total calcium levels were estimated in sciatic nerve as described by Severinghaus and Ferrebee, 1950 [32]. Sciatic nerve homogenate was mixed with 1 ml of trichloroacetic acid (4%) in ice-cold conditions and centrifuged at 200 rpm for 10 minutes. The clear supernatant was used for the estimation of total calcium spectrophotometrically (UV-1700 Spectrophotometer, Shimadzu, Japan) against blank at 556 nm. The values of total calcium are expressed in ppm/mg of protein. All results are expressed as mean ± SEM of n (number of animals studied) observations. The data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons using the Graph pad prism 5 software. The p value <0.05 was considered to be statistically significant.

RESULTS

The baseline paw withdrawal response in each test obtained on day 0 for each rat was relatively stable and showed no significant variation. Following surgery, the rats kept their nerve injured paw elevated above the cage floor but otherwise appeared healthy, exhibited normal grooming and feeding behavior and gained weight normally. The ipsilateral and contralateral paw responses to thermal and cold stimulation in sham-operated rats remained unchanged from baseline values throughout the entire observation period. The ipsilateral paw withdrawal response of all the CCI induced rats were significantly less than that of sham operated rats on day 3 onwards and reached steady state between days 7 and 21 after surgery indicating the development and maintenance of allodynia and hyperalgesia in a time dependent manner. However, the contralateral PWLs in response to cold stimulation did not later in CCI rats as compared to that of sham-operated rats.

Effects of *Murraya koenigii* on thermal hyperalgesia in CCI rats

Chronic constriction injury (CCI) od sciatic nerve resulted in significant development of noxious thermal hyperalgesia, indicated by decrease in left hind paw withdrawal threshold, after the 3rd day of surgery as compared to sham control. Administration of left extract of *Murraya koenigii* (MK 100, 200 and 400 mg/kg, *p.o.*, for 2 weeks) significantly and dose-dependently attenuated CCI induced decrease in the nociceptive threshold for thermal hyperalgesia. However, statistically significant attenuation was recorded only with medium and high dose of *Murraya koenigii* leaf extract. Lower dose of MK (100 mg/kg, *p.o.*) did not

show any significant effect on hyperalgesia as compared to CCI control group. Furthermore, to assess whether the discontinuation of *Murraya koenigii* leaf extract administration results in return of the behavioral symptoms of hyperalgesia or in the maintenance of antihyperalgesic effects, rats were submitted to nociceptive test during the next one week following the treatment discontinuation on day 14. The relief of hyperalgesia elicited by the high and middle dose of *Murraya koenigii* leaf extract (400 and 200 mg/kg, *p.o.*) sustained even after the discontinuation of the treatment.Treatment of pregabalin (10mg/kg, *p.o.*) significantly attenuated CCI induced hyperalgesia as compared to CCI control group. Further, *Murraya Koenigii per se* did not show any significant effect on thermal hyperalgesia (Fig. 1).

Effect of *Murraya koenigii* on cold allodynia in CCI rats

Chronic constriction injury of sciatic nerve resulted in significant development of non-noxious cold allodynia, indicated by decrease in left hind paw withdrawal threshold, after the 3rd day of surgery as compared to sham control (Fig. 2). Administration of leaf extract of *Murraya koenigii* (MK 100, 200 and 400 mg/kg, *p.o.*, for 2 weeks) significantly and dose-dependently attenuated the development of hypersensitivity to cold stimulation in ipsilateral hind paw of CCI rats as compared to CCI control rats. However, statistically, significant attenuation was recorded only with medium and high dose of *Murraya koenigii* leaf extract (200 and 400 mg/kg, *p.o.*). Lower dose of MK (100 mg/kg, *p.o.*) did not show any significant effect on cold allodynia as compared to CCI control group. In all treatment groups, administration of *Murraya koenigii* had no effect on the contralateral paw withdrawal response in this test as compared to that of CCI rats. Furthermore, to assess whether the discontinuation of *Murraya koenigii* leaf extract administration results in return of the behavioral symptoms of allodynia or in the maintenance of antiallodyic effects, rats were submitted to behavioral assays of neuropathic pain during the next one week following the treatment termination on day 14. The relief of allodynia elicited by the high and middle dose of *Murraya koenigii* leaf extract (400 and 200 mg/kg, *p.o.*) sustained even after the discontinuation of the treatment.Treatment of pregabalin (10mg/kg, *p.o.*) significantly attenuated CCI induced allodynia as compared to CCI control group. Further, *Murraya Koenigii per se* did not show any significant effect on cold allodynia.

Effect of *Murraya koenigii* on total protein

Total protein level was not affected significantly as aresult of chronic constriction injury of sciatic nerve as compared to sham operated rats (Table1).

Effect of *Murraya koenigii* on lipid peroxidation

CCI induced rats had significantly increased level of TBARS, an index of lipid peroxidation, in ipsilateral sciatic nerve as comparison to sham operated rats, assessed on day 21 following nerve injury (Table 1). Administration of the leaf extract of *Murraya koenigii* (100, 200 and 400 mg/kg, *p.o.*, for 2 weeks) significantly and dose-dependently reduced elevated levels of TBARS in the ipsilateral sciatic nerve of CCI rats in comparison to the levels observed in CCI control rats on day 21. Statistically significant attenuation was recorded only with the medium and high dose of MK leaf extract (200 and 400 mg/kg, *p.o.*).

Treatment with pregabalin (10 mg/kg, *p.o.*) significantly reduced the elevated level of TBARS in ipsilateral sciatic nerve of CCI induced rats as compared to CCI control group. Further, *Murraya koenigii per se* did not show any significant effect.

Effect of *Murraya koenigii* on reduced glutathione

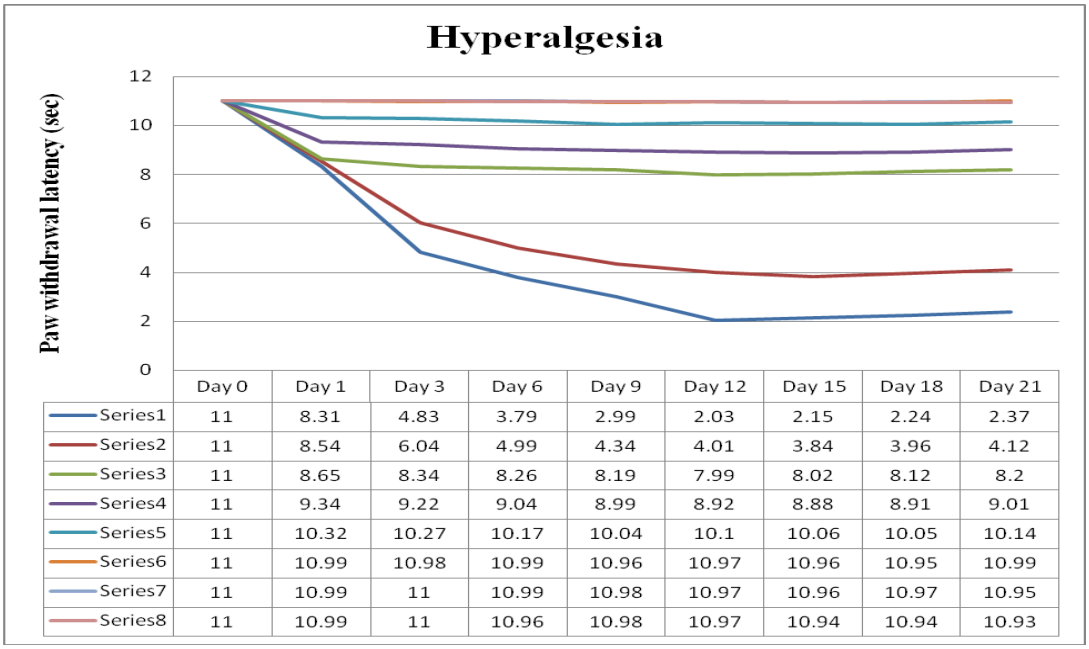
Nerve injury caused marked reduction in GSH level in ipsilateral sciatic nerve as compared to that of sham-operated rats (Table 2). Administration of the leaf extract of *Murraya koenigii* (100, 200 and 400 mg/kg, *p.o.*, for 2 weeks) significantly restored the depleted level GSH as compared to that of CCI control rats in a dose-dependent manner. However, lower dose of *Murraya koenigii* (100 mg/kg, *p.o.*) did not show any statistically significant effect in restoring the depleted level of GSH. Only the medium and high dose of *Murraya koenigii* leaf extract (200 and 400 mg/kg, *p.o.*) significantly improved the level of GSH in ipsilateral sciatic nerve of CCI rats as compared to CCI control group.

Treatment with pregabalin (0 mg/kg, *p.o.*) significantly recovered the depleted level of GSH in ipsilateral sciatic nerve of CCI induced rats as compared to CCI control group. Further, *Murraya koenigii per se* did not show any significant effect.

Effect of *Murraya koenigii* on calcium level

Sciatic nerve ligation resulted in significant rise in total calcium levels noted after 21 days of surgery as compared to sham operated rats (Table 2). Administration of the leaf extract of *Murraya koenigii* (100, 200 and 400 mg/kg, *p.o.*, for 2 weeks) significantly attenuated CCI induced rise in total calcium level as compared to that of CCI rats in a dose-dependent manner. However, statistically significant attenuation was recorded only with medium and high dose of *Murraya koenigii* leaf extract (200 and 400 mg/kg, *p.o.*).

Treatment with pregabalin (10 mg/kg, *p.o.*) significantly reduced the elevated level of calcium in ipsilateral sciatic nerve of CCI induced rats as compared to CCI control group. Further, *Murraya koenigii per se* did not show any significant effect.



	Day 0	Day 1	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18	Day 21
Series1	11	8.31	4.83	3.79	2.99	2.03	2.15	2.24	2.37
Series2	11	8.54	6.04	4.99	4.34	4.01	3.84	3.96	4.12
Series3	11	8.65	8.34	8.26	8.19	7.99	8.02	8.12	8.2
Series4	11	9.34	9.22	9.04	8.99	8.92	8.88	8.91	9.01
Series5	11	10.32	10.27	10.17	10.04	10.1	10.06	10.05	10.14
Series6	11	10.99	10.98	10.99	10.96	10.97	10.96	10.95	10.99
Series7	11	10.99	11	10.99	10.98	10.97	10.96	10.97	10.95
Series8	11	10.99	11	10.96	10.98	10.97	10.94	10.94	10.93

Fig. 1: Effect of *Murraya koenigii* on thermal hyperalgesia assessed by the noxious thermal stimulus evoked ipsilateral hind paw withdrawal latency.

Series 1 = CCI

Series 2 = CCI + MK (100 mg/kg)

Series 3 = CCI + MK (200 mg/kg)

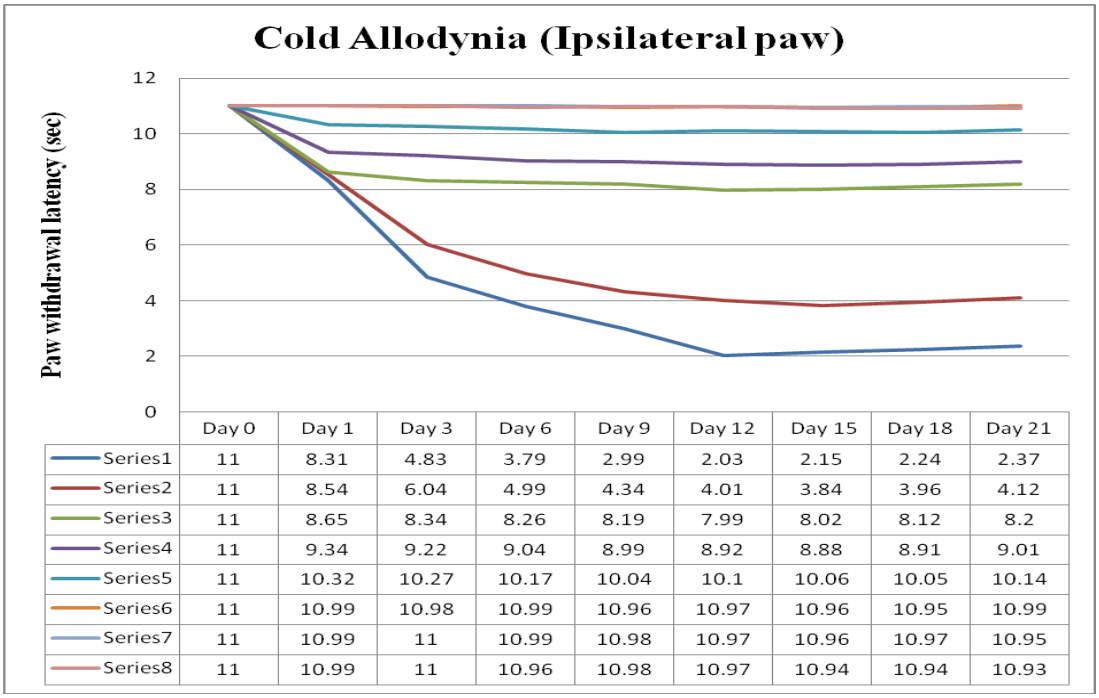
Series 4 = CCI + MK (400 mg/kg)

Series 5 = CCI + Pregabalin

Series 6 = MK *per se*

Series 7 = Sham

Series 8 = Normal



	Day 0	Day 1	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18	Day 21
Series1	11	8.31	4.83	3.79	2.99	2.03	2.15	2.24	2.37
Series2	11	8.54	6.04	4.99	4.34	4.01	3.84	3.96	4.12
Series3	11	8.65	8.34	8.26	8.19	7.99	8.02	8.12	8.2
Series4	11	9.34	9.22	9.04	8.99	8.92	8.88	8.91	9.01
Series5	11	10.32	10.27	10.17	10.04	10.1	10.06	10.05	10.14
Series6	11	10.99	10.98	10.99	10.96	10.97	10.96	10.95	10.99
Series7	11	10.99	11	10.99	10.98	10.97	10.96	10.97	10.95
Series8	11	10.99	11	10.96	10.98	10.97	10.94	10.94	10.93

Fig. 1: Effect of *Murraya koenigii* on cold allodynia assessed by the non-noxious thermal stimulus evoked ipsilateral hind paw withdrawal latency.

Series 1 = CCI
Series 2 = CCI + MK (100 mg/kg)
Series 3 = CCI + MK (200 mg/kg)
Series 4 = CCI + MK (400 mg/kg)
Series 5 = CCI + Pregabalin
Series 6 = MK *per se*
Series 7 = Sham
Series 8 = Normal

Table 1: Effect of *Murraya koenigii* on CCI induced alterations in total protein level and TBARS.

Groups	Protein (mg/ml)	MDA (nmole/mg of protein)
Normal	4.24 ± 0.31	3.11 ± 0.36
Sham	4.19 ± 0.55	3.18 ± 0.22
CCI	4.71 ± 0.59[a]	4.91 ± 0.36[a]
MK *per se*	4.15 ± 0.34	3.13 ± 0.37
CCI + MK (100 mg/kg)	4.65 ± 0.41[a]	4.72 ± 0.42[a]
CCI + MK (200 mg/kg)	4.58 ± 0.41[b]	3.47 ± 0.20[b]
CCI + MK (400 mg/kg)	4.40 ± 0.31[b]	3.30 ± 0.16[b]
CCI + Pregabalin	4.28 ± 0.39[b]	3.23 ± 0.29[b]

[a]$p < 0.05$ vs. sham control group

[b]$p < 0.05$ vs. CCI control group

Table 2: Effect of *Murraya koenigii* on CCI induced alterations reduced GSH and calcium levels.

Groups	GSH (microgm/mg of protein)	Calcium (ppm/mg of protein)
Normal	72.93 ± 1.27	4.03 ± 1.16
Sham	72.36 ± 1.09	3.95 ± 0.73
CCI	45.46 ± 1.62[a]	35.43 ± 1.51[a]
MK *per se*	73.34 ± 1.12	3.87 ± 0.64
CCI + MK (100 mg/kg)	51.64 ± 1.49[a]	26.26 ± 1.61[a]
CCI + MK (200 mg/kg)	64.02 ± 1.11[b]	11.59 ± 1.34[b]
CCI + MK (400 mg/kg)	68.58 ± 1.82[b]	8.25 ± 0.29[b]
CCI + Pregabalin	70.49 ± 1.50[b]	4.43 ± 1.91[b]

[a]$p < 0.05$ vs. sham control group

[b]$p < 0.05$ vs. CCI control group

DISCUSSION

The results of the present study demonstrate that oral administration of *Murraya koenigii* significantly alleviated the behavioral (i.e., thermal hyperalgesia and cold allodynia) and biochemical (i.e., reduced glutathione, lipid peroxidation, total protein and total calcium level) changes caused by chronic constriction injury of sciatic nerve in rats. Neuropathic pain is a chronic condition which is characterized by the sensory symptoms like hyperalgesia and allodynia. CCI nerve injury produces robust thermal hyperalgesia and cold allodynia in the ipsilateral paw, start within 3-5 days of the post-nerve injury and lasts throughout the experimental period [33-34]. Also, in the present study it is observed that the ipsilateral paw withdrawal latencies (PWL) of CCI-control rats showed marked thermal hyperalgesia and cold allodynia. The present study resulted in MDA (an index of lipid peroxidation), total calcium levels rise and fall in the reduced glutathione (GSH, an endogenous anti-oxidant) level in CCI induced rats. Thus, supporting the contention that free radicals may contribute in the pathogenesis of neuropathy.

In this investigation, CCI induced behavioral and biochemical changes have been attenuated by administration of *Murraya koenigii* leaf extract. *Murraya koenigii* is previously reported to possess antioxidant, anti-inflammatory, analgesic, anti-lipid peroxidative and many other activities [24]. *Murraya koenigii* is rich in carbazole alkaloids (Mahanimbine and koenigine), phenolics, polyphenols and flavonoids [35-37]. The results of various *in vitro* experiments reveal that the ethanolic extract possess DPPH radical scavenging activity, reducing power, superoxide anion free radical scavenging property and –OH scavenging activities [37-38]. These studies indicate that the *Murraya koenigii* leaf extract contains antioxidant

phytonutrients such as mahanimbine and koenigine which are highly effective and functions synergistically *in vivo* providing prevention against CCI induced oxidative damage. Oral administration of *Murraya koenigii* to CCI induced rats also decreased elevated calcium level. Therefore, the observed decrease in calcium levels with *Murraya koenigii* may possibly be attributed to its anti-oxidant effects. However, it may also be possible that direct action of *Murraya koenigii* is responsible for the decrease in the calcium levels in CCI induced animals. Therefore, the observed decrease in calcium levels may be either due to direct action or secondary to decrease in oxidative stress.

In the present investigation, pregabalin was used as a standard drug. Pregabalin [(S)-3-(aminomethyl)-5-methylhexanoic acid or S-(+)-isomer of 3-isobutyl gamma-aminobutyric acid] is an anti-convulsant that successfully treats many neuropathic pain syndromes [39]. Pregabalin is a selective Ca_v 2.2 (alpha2-delta subunit) channel antagonist. It is a structural analogue (but not functional) of the gamma aminobutyric acid. Pregabalin has analgesic, anti-convulsant and anxiolytic activities [40]. Preclinical trials have demonstrated an anti-hyperalgesic and anti-allodynic effect of pregabalin in various animal models of neuropathic pain [39, 41-42].

Since pregabalin is well documented to exert its beneficial effect in neuropathic pain and *Murraya koenigii* has also been shown to alleviate CCI induced behavioral and biochemical changes. Therefore, it may be concluded that potential anti-oxidant, anti-inflammatory, neuroprotective and calcium level modulatory actions of *Murraya koenigii* are responsible for attenuating CCI induced peripheral neuropathic pain.

CONCLUSION

The present study was designed to investigate the possible therapeutic effect of *Murraya koenigii* in treatment of chronic constriction injury (CCI) of sciatic nerve induced peripheral neuropathic pain. A marked thermal hyperalgesia and cold allodynia from day 3 onwards was observed which continued to develop for 14 days and maintained for the next 1 week. This showed the existence of neuropathic pain. Increased level of TBARS, depleted level of GSH and an increased level of calcium was observed. Administration of *Murraya koenigii* leaf extract (100, 200 or 400 mg/kg, *p.o.*, once daily) till 14th day after surgery improved thermal hyperalgesia, cold allodynia, decreased CCI-induced raised levels of TBARS, an index of lipid peroxidation and restored the depleted levels of GSH (reduced glutathione). It also decreased the calcium levels. Thus, the results of the present study demonstrated that the

administration of ethanolic extract of *Murraya koenigii* leaves prevented the development and maintenance of peripheral neuropathic pain.

ACKNOWLEDGEMENT

The authors would like to extend their gratitude towards Dr. Anita Kotwani, Associate Professor, Vallabhbhai Patel Chest Institute, University of Delhi, for her motivation, support and sharing some extremely valuable ideas and suggestions without which this article would have been incomplete.

REFERENCES

1. C.J. Woolf, R.J. Mannion, *Lancet*, 1999; 353(9168): 1959-1964.
2. H. Merskey, N. Bogduk, *International Association for the Study of Pain*, 1994; 2: 209-214.
3. A.B. O'Connor, R.H. Dwarkin, *American Journal of Medicine*, 2009; 122: 22-32.
4. N. Torrance, B.H. Smith, M.I. Bennett, A.J. Lee, *J Pain*, 7(4): 281-289.
5. D. Bouhassira, M. Lanteri-Minet M, N. Attal, B. Laurent, C. Touboul, *Pain*, 2008; 136(3): 380-387.
6. B. Gustorff, T. Dorner, R. Likar, *Acta Anaesthesiol Scand*, 2008; 52(1): 132-136.
7. R.D. Treede, R.A. Meyer, S.N. Raja, J.N. Campbell, *Prog Neurobiol*, 1992; 38(4): 397-421.
8. I. Gilron, C. Peter, N. Watson, C.M. Cahill, D.E. Moulin, *Can Med Ass J*, 2006; 175: 265-275.
9. F.A. White, H. Jung, R.J. Miller, *PNAS*, 2007; 104(51): 20151-20158.
10. H.H. Kramer, L. He, B. Lu, F. Birklein, C. Sommer, *Neurobiol Dis*, 2009; 35(2): 177-183.
11. H. Kurvers, M. Daemen, D. Slaaf, F. Stassen, F. van der Wildenberg, P. Kitslaar, J. Mey, *Acta Orthop Belg*, 1998; 64(1): 64-70.
12. R.M. Nagler, *Neurology*, 2010; 75(2): 109-110.
13. H.J. McQuay, M. Tramer, B.A. Nye, D. Carroll, P.J. Wiffen, R.A. Moore, *Pain*, 1996; 68(2-3): 217-27.
14. M. Davis, *Support Care Cancer*, 2007; 15(4): 363-372.
15. D.E. Moulin, A.J. Clark, I. Gilron, M.A. Ware, C.P. Watson, B.J. Sessle, *Pain Res Manag*, 2007; 12: 13-21.
16. S. Arner, B.A. Meyerson, *Pain*, 1988; 33(1): 11-23.

17. S. Babbar, J.F. Marier, M.S. Mouksassi, M. Beliveasu, G.F. Vanhove, S. Chanda, *Ther Drug Monit*, 2009; 31(4): 502-510.
18. S.M. Hiremath, B.B. Mdalageri, P.W. Basarkar, *Indian Perfum*, 1998; 42: 58-59.
19. M. Omankutty-Amma, K. Rajaraman, B. Sankarikutty, M.A. Sumathikutty, K.P. Padmakumari, C.S. Narayanan, *Indian Food Packer*, 1984: 32-36.
20. A. Ghani, *Dhaka Asiatic Society of Bangladesh*, 2003; 2: 309-310.
21. K.J. Shah, A.R. Juvekar, *Indian J Exp Biol*, 2006; 44(6): 481-4.
22. K. Srinivasan, *Int J Food Sci Nutr*, 2005; 56(6): 399-414.
23. P. Arulselvan, G.P. Senthilkumar, S.D. Kumar, S. Subramanian, *Pharmazie*, 2006; 61(10): 874-877.
24. D. Iyer, P.U. Devi, *Pharmacognosy Reviews*, 2008; 2: 180-184.
25. G.J. Bennett, Y.K. Xie, *Pain*, 1988; 33: 87-107.
26. N.B. Eddy, C.F. Touchberry, J.E. Lieberman, *J Pharmacol Exp Ther*, 1950; 98(2): 121-137.
27. S.S.V. Padi, S.K. Kulkarni, *Pharmacol Biochem Behav*, 2004; 79(2): 349-358.
28. N. Attal, G. Cruccu, R. Baron, *Eur J Neurol*, 2010; 10: 1468-1331.
29. W.G. Niehaus, D. Samuelsson, *Eur J Biochem*, 1968; 6(1): 126-130.
30. O.H. Lowry, N.J. Rosebrough, A.L. Farr, R.J. Randall, *J Biol Chem*, 1951; 193(1): 265-275.
31. G.L. Ellman, *Arch Biochem Biophys*, 1959; 82(1): 70-74.
32. J.W. Severinghaus, J.W. Ferrebee, *Journal of Biological Chemistry*, 1950; 187(2): 621-630.
33. S.S.V. Padi, S.K. Kulkarni, *Eur J Pharmacol*, 2008; 601(1-3): 79-87.
34. J. Xu, P.D. Drew, *J Neuroimmunol*, 2006; 171(1-2): 135-44.
35. U.P. Singh, D.P. Singh, S. Maurya, R. Maheshwari, M. Singh, R.S. Dubey, R.B. Singh, *J Herb Pharmacother*, 2004; 4(4): 27-42.
36. A.P.Singh, T. Wilson, V. Vorsa, D. Luthria, M.R. Freeman, R.M. Scott, D. Bilenker, S. Shah, S. Somasundaram, N. Vorsa, *Food Chem*, 2011; 127: 80-85.
37. M.B. Ningappa, R. Dinesha, L. Srinivas, *Food Chem*, 2008; 106: 720-728.
38. S. Sathaye, Y. Bagul, S. Gupta, H. Kaur, R. Redkar, *Exp Toxic pathol*, 2011; 63(6): 587-591.
39. N. Kumar, A. Laferriere, J.S. Yu, A. Leavitt, T.J. Coderre, *J Neurochem*, 2010; 113(2): 552-561.
40. P.Stump, *Drugs Today (Barc)*, 2009; 45(Suppl C): 19-27.

41. G. Bender, J.A. Florian Jr., S. Bramwell, M.J. Field, K.K. Tan, S. Marshall, J. DeJongh, R.R. Bies, M. Danhof, *J Pharmacol Exp Ther*, 2010; 334(2): 599-608.
42. H.J. Park, H.S. Joo, H.W. Chang, J.Y. Lee, S.H. Hong, Y. Lee, D.E. Moon, *Can J Anaesth*, 2010; 57(7): 664-671.