IJPSR (2015), Vol. 6, Issue 12 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 08 June, 2015; received in revised form, 21 July, 2015; accepted, 03 October, 2015; published 01 December, 2015

EFFECT OF ACAMPROSATE IN EXPERIMENTAL MODELS OF PERIPHERAL NEUROPATHIC AND INFLAMMATORY PAIN IN WISTAR RATS

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Keywords:

Neuropathic pain, Inflammatory pain, Acamprosate, Hyperalgesia, Allodania

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ABSTRACT: Neuropathic pain is a persistent chronic pain caused by primary lesion or dysfunction of neurons which is primarily characterized by hyperalgesia, allodynia. There is some evidence that acamprosate might inhibit Ca2 influx through NMDA and voltage-dependent Ca2 channels. λ-carrageenan (0.1 ml of 1% w/v) into i.pl. region of the hind paw was injected to produce acute inflammation. Unilateral peripheral mononeuropathy was induced by sciatic nerve ligation. Thermal and mechanical hyperalgesia and cold allodynia were assessed. The extent of oxido-nitrosative stress was assessed by estimating the levels of thiobarbituric acid reactive substances, catalase and superoxide dismutase activity and nitrite levels in both sciatic nerve and dorso-lumbar spinal cord homogenate in CCI model. The one hour pre-treatment with acamprosate (100 and 200 mg/kg, p.o) was given. Then second dose acamprosate treatment at the 19 hr post-carrageenan given after first dose oral administration of acamprosate at a dose of 200 mg/kg., but not 100 mg/kg significantly reversed the established mechanical hyperalgesia without producing significant effect on thermal hyperalgesia. The two week repeated administration of acamprosate (100 and 200 mg/kg, p.o.) significantly reversed the established thermal and mechanical hyperalgesia and cold allodynia. Thus, single dose treatment with acamprosate did not produce significant anti-inflammatory and anti-hyperalgesic effects, in carrageenan-induced inflammatory pain model. While, repeated administration of acamprosate ameliorates the behavioural symptoms of neuropathic pain observed CCI-induced NP. The observed effects may be partly due to activation of anti-oxidant mechanisms, but independent of anti-inflammatory effects.

INTODUCTION: Neuropathic pain (NP) is a chronic debilitating condition. The International association for study of pain (IASP) has defined neuropathic pain (NP) as "pain initiated or caused by a primary lesion or dysfunction in the nervous system" [1]. NP is often characterized by stimulus-independent persistent pain or abnormal sensory perception of pain such as allodynia and hyperalgesia [2, 3].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.6(12).5102-14

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.6(12).5102-14

NP can be caused by trauma (e.g., spinal cord injury, stroke), disease conditions (e.g., diabetic neuropathy, HIV-associated neuropathy) and major surgeries (e.g., thoracotomy, amputation) and anticancer agents including vincristine and paclitaxel [3]. Currently available drugs for neuropathic pain include antidepressants, anticonvulsants, sodium and calcium channel blockers, N-methyl-D-aspartic acid (NMDA) receptor antagonists and opioids. These drugs, however, provide a transient relief of neuropathic pain, in only a fraction of patients and they often produce severe CNS related, dose-limiting, side effects [4, 5].

The mechanism(s) underlying neuropathic pain are not completely understood but are considered to be

complex, multifactorial and to evolve over time. Current research studies indicate that both peripheral and central mechanisms are involved in pathogenesis of neuropathic pain [6, 7]. Peripheral nerve injury is associated with a local inflammatory reaction of the nerve trunk and the released inflammatory mediators sensitize the axotomized nerve fibers [8, 9, 10.]

The functional N-methyl-D-aspartate receptor (NMDAR) is a heteromeric complex containing NR1 and NR2 subunits. The NR1 protein is an obligatory component for a functional NMDAR, with at least one of the NR2 subunit family member, of which NR2A and NR2B are the most abundant in adult rat dorsal horn [11]. Although the precise mechanism of chronic pain after spinal cord injury remains elusive, considerable evidence indicates that NMDAR in the superficial dorsal horn has been implicated as a major contributor to excitatory nociceptive transmission [12, 13] and also essential for central sensitization [14].

It is now clearly established that NP is associated with activation and/or modulation of multiple pathogenic mechanisms. Thus, there is an unmet need to characterize and develop newer agents, which are act at more than one site to effectively alleviate the symptoms and pathogenesis of NP. Acamprosate (calcium bis acetyl-homotaurine) is EU anf FDA approved medicine for the prevention of relapse in alcoholics [15]. Acamprosate is the calcium salt of N-acetyl homotaurine, a small, highly flexible molecule with similarities to many amino acids, most notably glutamate, gamma-aminobutyric acid, aspartate, glycine, and taurine[15, 16]. Acamprosate has similar effects, as compared to anti-oxidants on oxygen free radicals during alcohol intoxication [17].

Radioligand binding studies suggested that acamprosate is act as a weak partial agonist effects on NMDA receptors through indirect actions on a polyamine site on the NMDAR complex [18, 19]. Other studies have suggested that acamprosate might interact with the polyamine site of the NMDA receptor [19, 20, 21]. In addition, there is some evidence that acamprosate might inhibit Ca^2 influx through NMDA and voltage-dependent Ca^2 channels [22].

This study was designed to investigate the effect of Acamprosate in Experimental Models of Peripheral Neuropathic and Inflammatory Pain in wistar Rats.

MATERIALS AND METHODS:

The experiment was performed at ISF College of pharmacy, Moga, Punjab and was performed in year 2013.

Experimental animals:

Wistar rats, weighing 170-250gm, were used in present study. They were housed in Central Animal House facility of ISF College of Pharmacy, Moga, Punjab, India, in group of three, in polypropylene cages with husk bedding under standard conditions of light and dark cycle with food and water ad libitum. Animals were acclimatized to laboratory conditions before the test. All the behavioral assessments were carried between 8:00 hrs and 16:00 hrs.

TABLE: EXPERIMENTAL PROTOCOL

Experimental groups	Treatment
I	Vehicle treated carrageneen control
II	Vehicle treated CCI-control
III	Carragenan + Acamprosate (100 mg/kg, p.o)
IV	Carragenan + Acamprosate (200 mg/kg, p.o)
V	Carragenan + Diclofenac (20 mg/kg, p.o.)
VI	CCI + Acamprosate (100 mg/kg, p.o)
VII	CCI +Acamprosate (200 mg/kg, p.o)
VIII	CCI+ Pragabalin (30 mg/kg, p.o)

Chronic constrictive injury (CCI) of sciatic nerve-induced neuropathic pain:

The mononeuropathy was produced according to the method described by Bennett and Xie (1988) [23]. Briefly, the rats were anesthetized using thiopental sodium (40 mg/kg i.p.) and the common sciatic nerve of the left hind paw was exposed at the level of the middle of the thigh by blunt dissection through the biceps femoris muscle. Proximal to the sciatic trifurcation, approximately 7-mm of nerve was freed and 4 ligatures of 4-0 silk suture were placed around the sciatic nerve with 1mm interval. Great care was taken not to interrupt epineual blood flow during tying the ligature. In sham-operated rats, the same surgical procedure was followed, the connective tissue was freed, and no ligatures were

applied. After surgery, all animals received gentamycin (5 mg/kg, i.p.) to prevent sepsis.

Carrageenan-induced inflammatory pain model:
The inflammatory pain was induced in wistar rats by injecting 0.1 ml of 1% solution of λ-carrageenan (sigma-aldrich) into the intra planter region of the hind paw under light ether anesthesia [24]. The extracts were administered 2 hours after the injection of carrageenan. The degree of inflammation was assessed by measuring the volume of paw by measuring the volume of hind paw by using plethysmograph (INCO, Ambala) before (basal) and after 1, 2, 3, 5, 20, 21 and 23 hr after carrageenan administration in all the groups. Thermal hyperalgesia was assessed by recording the withdrawal latency of hind paw before (basal), 2.30 and 4.30 hr after carrageenan injection. Mechanical allodynia was assessed by measuring the mechanical threshold (gm) before (basal) and 2.15, 4.15, 20.15, 22.15 hr and 23.15 hr post-carrageenan.

Treatment schedule:
All the animals were acclimatized to laboratory environment for at least 3 days before taking basal behavioral reading. To evaluate the effect of acamprosate (100 and 200 mg/kg) in CCI model treatment was started on day 7 after nerve injury and was continued up to next 14 days days.

Assessment of Thermal hyperalgesia:
Hyperalgesia to thermal stimulation was determined using a Plantar Test Apparatus (Ugo Basile, Comerio, Italy) modeled as described by Hargreaves *et al*. (1998) [24]. In brief, rats were placed individually in Plexiglas cubicles mounted on a glass surface maintained at 25±0ºC. During this time, the rats initially demonstrated exploratory behavior, but subsequently stopped exploring and stood quietly with occasional bouts of grooming. A thermal stimulus, in the form of radiant heat emitted from a focused projection bulb, which was located under the glass floor, was focused onto the plantar surface of the left hind paw, and paw withdrawal latencies (PWLs) were recorded at interval of 15min and the mean of the three values was used for analysis. The intensity of radiant heat was adjusted to give 18–19sec withdrawal latency in rats. A cut-off latency of 20sec was set to avoid tissue damage. The response latency was determined using a timer linked to the photodiode motion sensors in the plantar reflex device.

Assessment of mechanical allodynia:
The threshold for touch sensitivity was measured in both hind paws, using an automated apparatus for applying reproducible light touch (Dynamic plantar Aesthesiometer 37400-002; Ugo Basile, Comerio, Italy). Animals were placed in their compartments on the metal mesh surface. After a short period, in which they showed exploratory behavior, they remained still in a resting position and at this time the test began. With the help of an adjustable angled mirror, the touch stimulator unit was placed beneath the selected hind paw to position the filament below the plantar surface of the animal. When the unit is started the electrodynamics actuator lifts the stainless steel filament, which touches the plantar surface and begins to exert an upward force below the threshold of feeling. The force increases, until the animal moves its paw or until the point at which greatest present force is met. The maximum value of force in grams (50 g) was previously fixed [25].

Assessment of cold allodynia in rats:
Cold allodynia was measured as the number of foot withdrawal responses after application of Cold stimuli to the planter surface of the paw. Age matched control and diabetic animals were gently restrained and both the hind paws were immersed on cold water (4-6ºC) for a period of 15secs (cut off time) [26] . Paw withdrawal latency for each hind paw was measured and the experiment was repeated 3 times for each rat. Paw-withdrawal latency was expressed as threshold levels in seconds.

Measurement of Locomotor activity:
Body weight and locomotor activity (motor behavior) were measured before and after induction of neuropathic pain on different days. The locomotor activity (horizontal activity) was measured by using an actophotometer. The locomotoractivity was expressed in terms of total photo beam interruption counts/5 min per animal [25].

Collection of tissue samples in rats: In this study, at the end of treatment schedule on day 28, animals

were sacrificed by cervical dislocation immediately after behavioral assays, followed by collection of sciatic nerve and spinal cord for estimation of markers of oxidative stress. Each part of sciatic nerve and spinal cord was washed with sterile normal saline. Weighed, homogenized in phosphate buffer pH 7.0, and centrifuged for 15min at 2000g to obtain the clear supernatant for the estimation of oxidative stress markers.

Estimation of lipid peroxidation in sciatic nerve and spinal cord: Concentration of thiobarbituric acid reactive substances (TBARS) was determined as an index of lipid peroxidation as described by Niehius and Samuelson (1968) [27]. In this method, 0.1 mL of sciatic nerve supernatant was treated with 2 mL of (1:1:1) 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 (0.25N). Then the mixture was boiled for 15 min, followed by centrifugation at 1000g for 10 min. Finally the absorbance was measured at 532 nm (UV-1700 Spectrophotometer, Shimadzu, Japan) against blank. Finally the values are expressed as n mole per g tissue.

Estimation of reduced glutathione in sciatic nerve and spinal cord: The concentration of endogenous antioxidant reduced glutathione (GSH) level in the sciatic nerve was estimated following the method described by Lou *et al.*(1988). In this method, 0.2 mL of supernatant was mixed with 1.78 mL of 1.0 M Tris buffer (pH 8.2) with 0.02 M ethylenediaminetetrachloroacetic acid (EDTA). Then 20µL of 0.1 M DTNB (5,5`-dithio-bis-2-nitrobenzoic acid, Ellman`s reagent) was added to the mixture and absorbance was noted at 412 nm (UV-1700 Spectrophotometer, Shimadzu, Japan) and the values expressed as µmole/ g tissue.

Measurement of nitrite in sciatic nerve and spinal cord

The nitrite concentration in the serum was measured by Griess reaction[28]. In this method, 0.1 Ml of supernatant of the nerve homogenate was mixed 0.25 mL of 1% sulfanilamide (prepared in 3 N HCL) and 0.25 mL of 0.1% N-(1-naphthyl) ethylene diamine dihydrochloride with shaking. After 10 min, absorbance was measured at 545 nm (UV-1700 Spectrophotometer, Shimadzu, Japan); the values of nitrite concentration were obtained from sodium nitrite standard curve, and are expressed in nmole/g tissue.

Measurement of Antioxidant Enzyme levels (Superoxide dismutase (SOD) in sciatic nerve and spinal cord: SOD was assessed by utilizing the method of Kakkar and Vishwnathan (1984) [29]. A single unit of enzyme was expressed as 50% inhibition of nitroblutetrazolium (NBT) reduction/min/mg protein by superoxide is measured at 560nm. In Brief, a mixture (1.5 ml) contained 1.0 ml of 0.01M phosphate buffer pH 7.0, 0.2 ml of tissue homogenate and 0.4 ml of 2M H_2O_2 . The reaction was stopped by the addition of 2.0 ml of dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic acid were mixed in 1:3 ratio). Results were expressed as SOD Enz. required for 50% inhibition of NBT reduction (% activity).

Statistical analysis:

The results are expressed as Mean ± S.E.M. The behavioral data were analyzed using two-way analysis of variance (ANOVA) followed by Bonferroni post hoc test for multiple comparison. $p < 0.05$ was considered statistically significant. For biochemical parameters one way analysis of variance (ANOVA) followed by Tukey post hoc test is used for comparison.

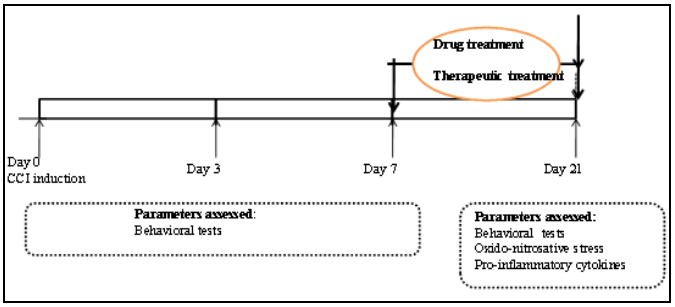

FIG.A: TREATMENT SCHEDULE IN CCI MODEL

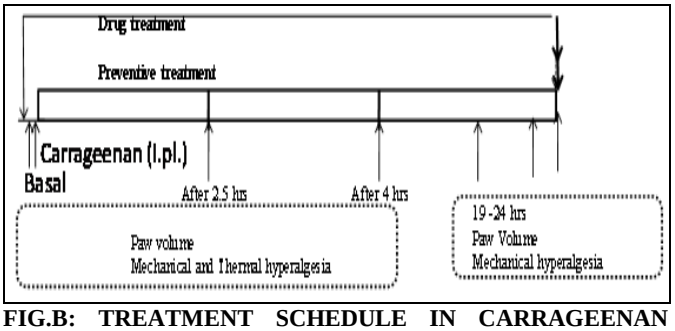

FIG.B: TREATMENT SCHEDULE IN CARRAGEENAN MODEL

RESULT: The baseline paw withdrawal latencies (PWLs) in each test obtained on day 0 for each rat was relatively stable and showed no significant difference. Following surgery, the rats were kept with 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 PWL to mechanical, thermal and cold stimulation in sham operated animals remained unchanged from baseline values, throughout the observation period. The ipsilateral PWLs of a vehicle treated chronic nerve constriction injured (CCI) rats were significantly less than that of sham operated rats from day 3 onwards and reached steady-state between days 7 and 21 post-sciatic nerve ligation, indicating the development and maintenance of stable allodynia and hyperalgesia.

Effect of acamprosate on carrageenan-induced paw edema, mechanical and thermal hyperalgesia in rats:

The intraplantar (i.pl) administration of 1% carrageenan (0.1ml) has shown to produce the paw edema and mechanical hyperalgesia, which is persisted even at 24 hours after carrageenan control. The one hour pre-treatment with acamprosate (100 and 200 mg/kg, p.o) did not produce any effect on paw edema as well as thermal and mechanical hyperalgesia, as compared to vehicle treated carrageenan control. On the other hand, second dose oral administration of acamprosate at a dose of 200mg/kg., but not 100mg/kg at 19hr post-carrageenan significantly reversed the established mechanical hyperalgesia without producing significant effect on thermal hyperalgesia, as compared to vehicle treated carrageenan control. Conversely, treatment with diclofenac (20mg/kg, p.o.) significantly attenuated the carrageenan induced thermal hyperalgesia and allodynia as shown in **Fig. 1, 2** and **3**.

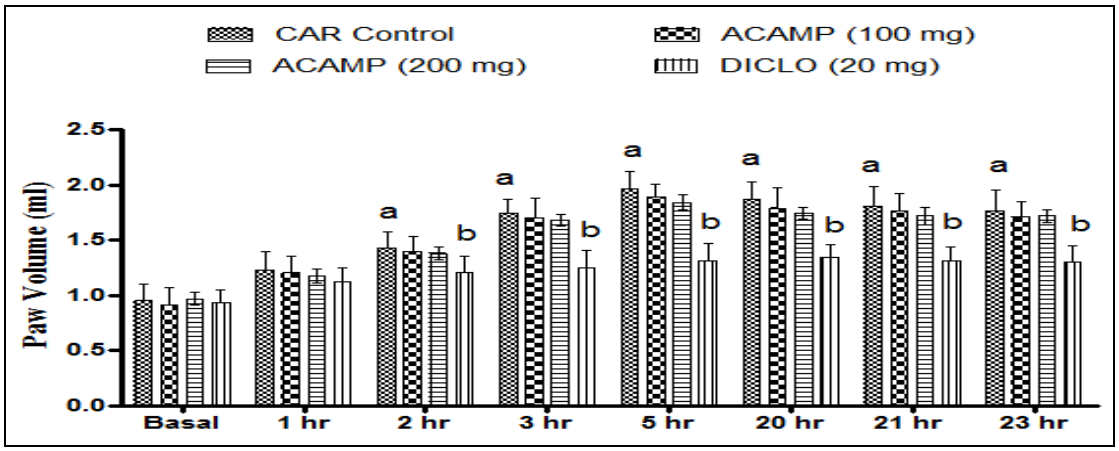

FIG.1: EFFECT OF ACAMPROSATE ON CARRAGEENAN-INDUCED PAW EDEMA.
All values are expresses as Mean ± S.E.M. a = p< 0.05 vs. Basal; b = p< 0.05 vs. CAR control.

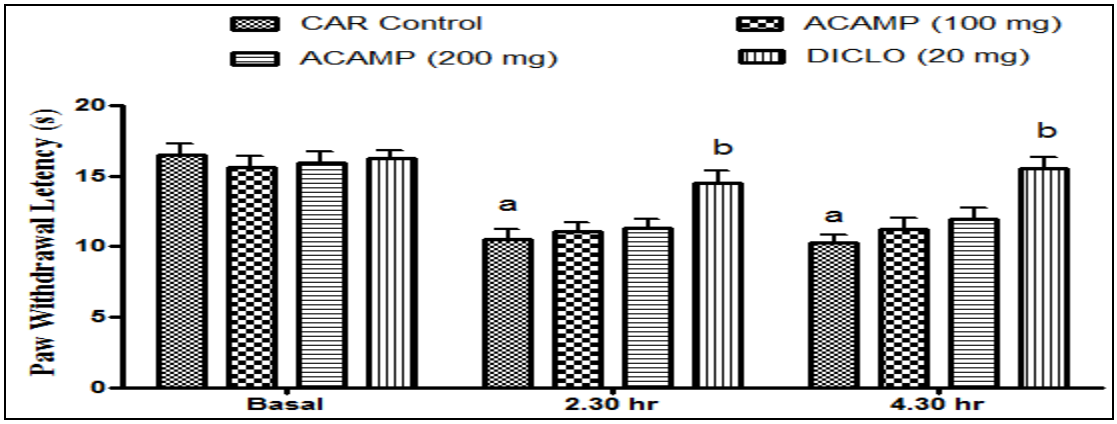

FIG.2: EFFECT OF ACAMPROSATE ON CARRAGEENAN-INDUCED THERMAL HYPERALGESIA.
All values are expresses as Mean ± S.E.M. a = p< 0.05 vs. Basal; b = p< 0.05 vs. CAR control.

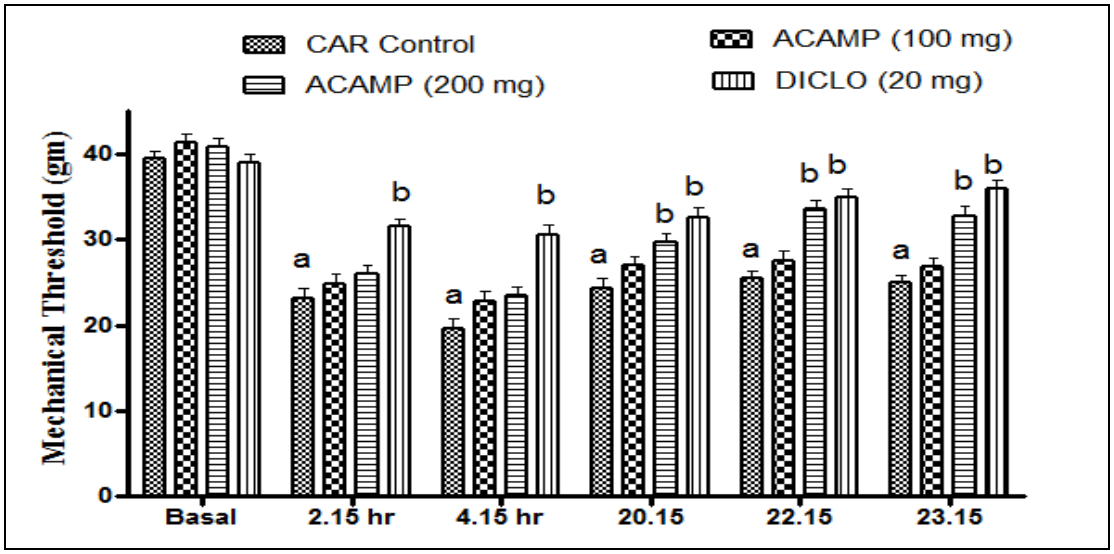

FIG.3: EFFECT OF ACAMPROSATE ON CARRAGEENAN-INDUCED MECHANICAL HYPERALGESIA.
All values are expresses as Mean ± S.E.M. a = p< 0.05 vs. Basal; b = p< 0.05 vs. CAR control.

Effect of single dose of acamprosate on established mechanical and thermal hyperalgesia in CCI rats:

The PWLs to cold stimuli in sham-operated animals during the entire observation period did not alter significantly from PWLs observed 1h before vehicle administration on day 7. CCI rats had shown decreased ipsilateral PWLs to cold stimuli as compared to that of sham group on day 7 following surgery indicate allodynia was established. Further, administration of vehicle to CCI rats did not alter allodynia throughout observation period. The ipsilateral PWLs in all nerve-injured animals were not significantly different on day 7. Administration of single dose of acamprosate (100 and 200 mg/kg, p.o.) had no effect on thermal hypergesia in CCI rats. On the other hand, acamprosate at a dose of 200 mg/kg, p.o., significantly increased ipsilateral mechanical threshold in CCI rats at 2 and h after its administration on day 7 as compared to PWLs at 0 h indicates reversal of established mechanical hyperalgesia. Conversely, pregabalin (30 mg/kg, p.o.) significantly increased ipsilateral PWLs to both thermal and mechanical stimuli in CCI rats at 2 and 4 h after its administration on day 7 as compared to PWLs at 0 as shown in **Fig.4** and **5**.

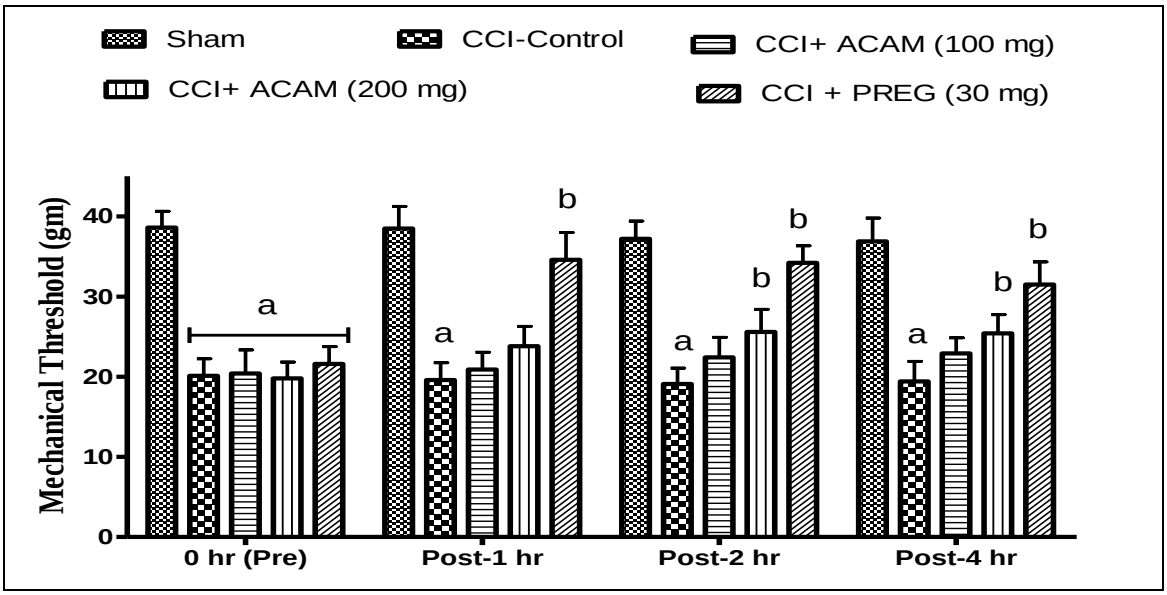

FIG. 4: ACUTE EFFECT OF ACAMPROSATE ON MECHANICAL HYPERALGESIA IN CCI RATS.
All values are expresses as Mean ± S.E.M. a = p< 0.05 vs. Basal; b = p< 0.05 vs. CAR control.

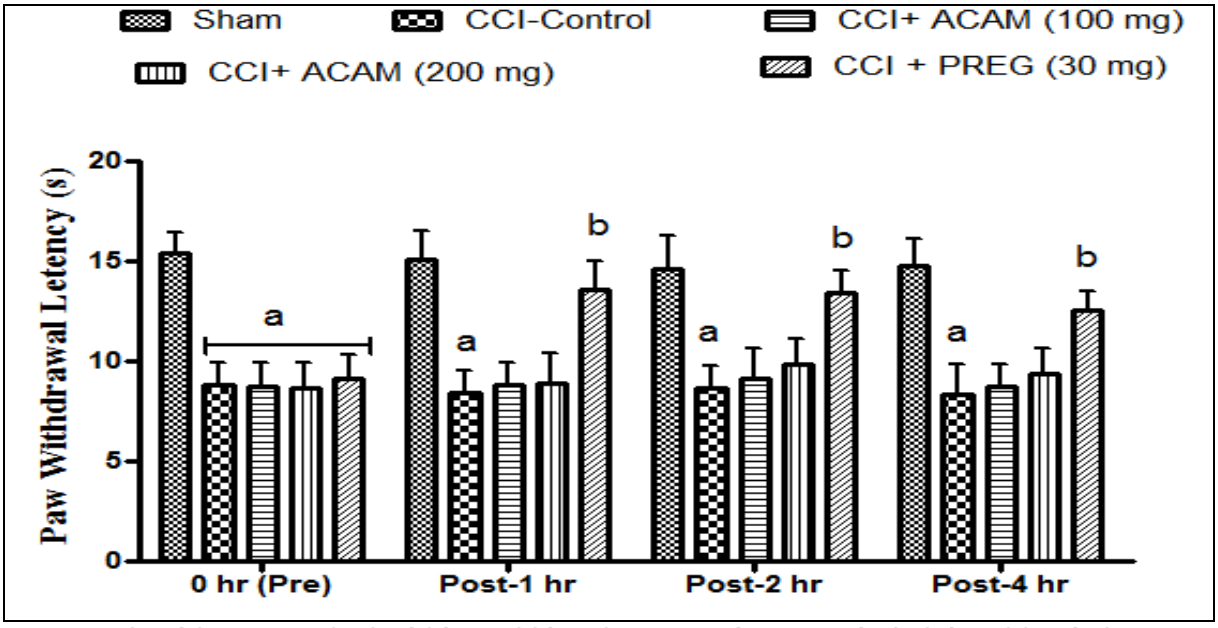

FIG.5: ACUTE EFFECT OF ACAMPROSATE ON THERMAL HYPERALGESIA IN CCI RATS.
All values are expresses as Mean ± S.E.M. a = p< 0.05 vs. Basal; b = p< 0.05 vs. CAR control.

Effect of repeated administration of acamprosate on mechanical and thermal hyperalgesia in CCI rats:

The PWLs to thermal and mechanical stimuli in sham-operated animals during the entire observation period did not differ significantly from basal PWLs. Repeated administration of acamprosate (100 and 200 mg/kg, p.o., for 2 weeks) significantly and dose-dependently reversed the reduced mechanical threshold (MT), as compared to vehicle-treated CCI rats. In the dosages used, the effect of standard drug, pregabalin was not significantly different from that produced by acamprosate (200mg/kg) on the day 21. Chronic administration of acamprosate (100 and 200 mg/kg, p.o., for 2 weeks) significantly and dose-dependently reversed the reduced ipsilateral PWL, as compared to vehicle-treated CCI rats. In the dosages used, the effect of standard drug, pregabalin was not significantly different from that produced by acamprosate (200mg/kg) on the day 21 as shown in **Fig 6 and 7**.

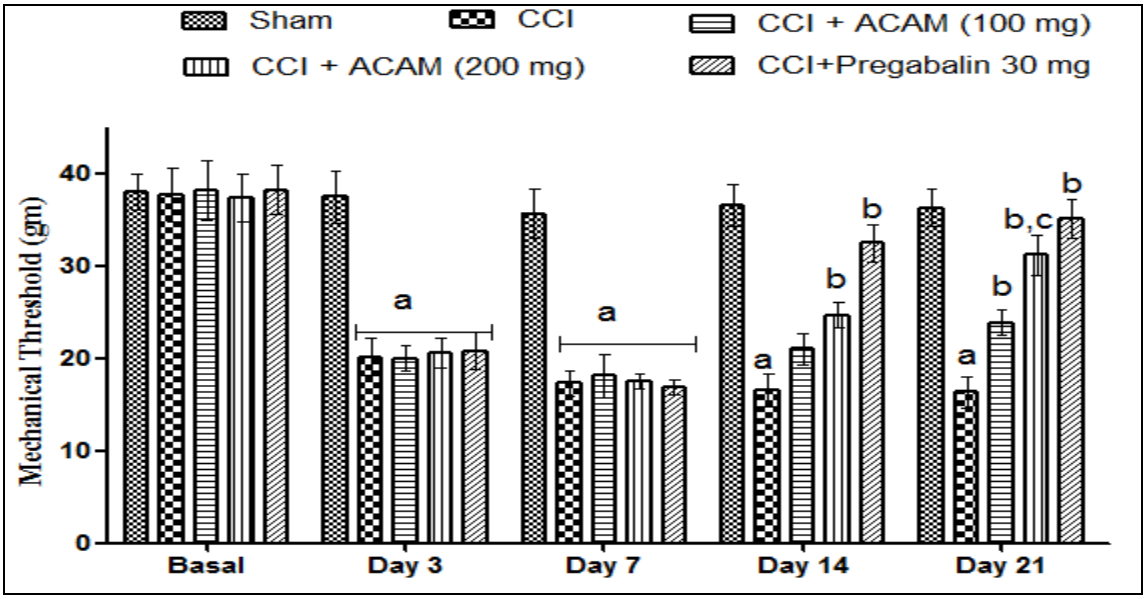

FIG.6: EFFECT OF REPEATED ADMINISTRATION OF ACAMPROSATE ON MECHANICAL HYPERALGESIA IN CCI RATS.
All values are expresses as Mean ± S.E.M. a = p< 0.05 vs. Basal; b = p< 0.05 vs. CAR control.

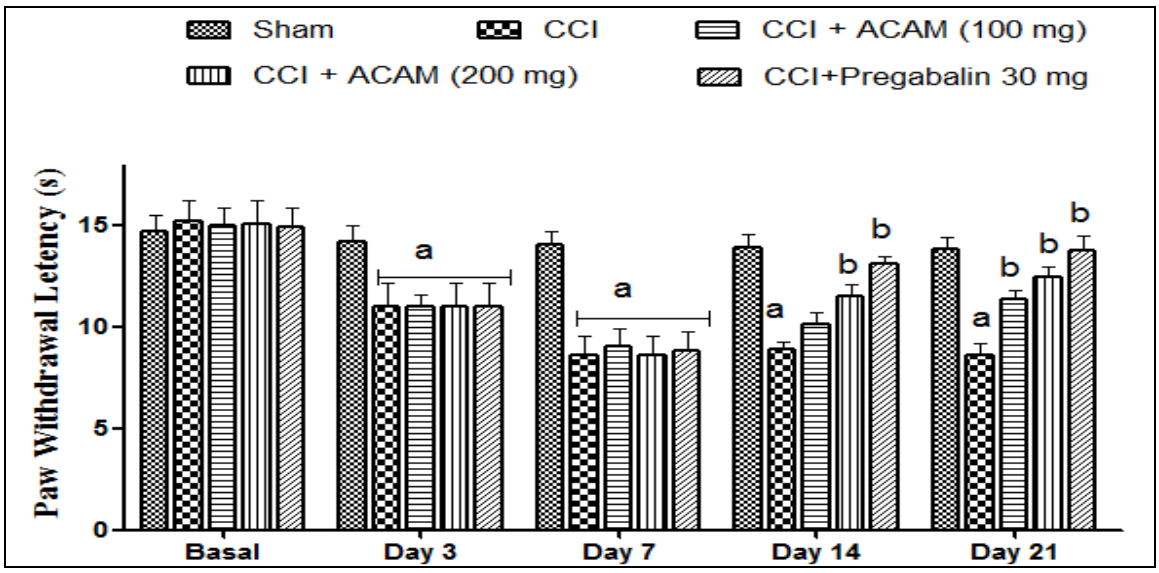

FIG.7: EFFECT OF REPEATED ADMINISTRATION OF ACAMPROSATE ON THERMAL STIMULI IN CCI RATS.
All values are expresses as Mean ± S.E.M. a = p< 0.05 vs. Basal; b = p< 0.05 vs. CAR control.

Effect of repeated administration of acamprosate on established cold allodynia in CCI rats:

CCI rats developed of allodynia, as shown by decreased ipsilateral PWLs in response to a cold stimulus, from day 3 onwards and further gets decreased towards week 2 indicating that neuropathic pain has developed as compared to that of sham-operated rats. Before treatment initiation, all the groups exhibited comparable baseline PWLs. Administration of acamprosate (100 and 200 mg/kg, p.o., for 2 weeks) significantly and dose dependently reversed the ipsilateral PWL, as compared to vehicle-treated CCI rats as shown in Fig 8.

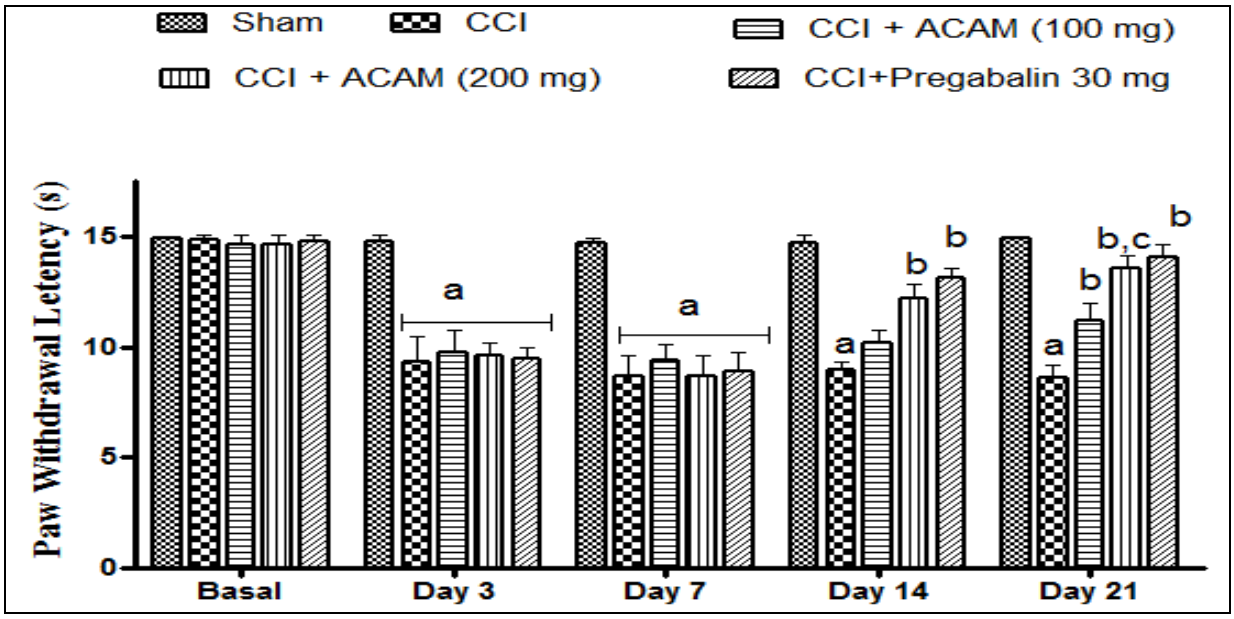

FIG. 8: EFFECT OF REPEATED ADMINISTRATION OF ACAMPROSATE ON COLD STIMULI IN CCI RATS.
All values are expresses as Mean ± S.E.M. a = p< 0.05 vs. Basal; b = p< 0.05 vs. CAR control.

Effect of repeated administration of acamprosate on ipsilateral sciatic nerve and spinal cord SOD, catalase, lipid peroxidation, and nitrite levels in CCI rats: The CCI in rats was associated with significant increase in ipsilateral sciatic nerve and spinal cord levels of TBARS, nitrite levels, but, significant decrease in the levels of superoxide dismutase (SOD) and catalase activity, as compared to sham-operated rats. The concentrations in the altered oxido-nitrosative stress parameters were significantly reversed in CCI rats treated with acamprosate (100 mg/kg, p.o.,

for two weeks), as compared with vehicle treated CCI control. The administration of standard drug, pregabalin and acamprosate were significantly, but partially on oxido-nitrosative stress parameters, while treatment with either pregabalin and/or acamprosate did not produce any significant effect on anti-oxidant enzymes, including SOD and catalase, as compared to vehicle treated CCI control group as shown in **Table 1** and **2**.

TABLE 1: EFFECT OF ORAL ADMINISTRATION OF ACAMPROSATE (100 and 200 mg/kg) ON SCIATIC NERVE SOD, CATALASE, LIPID PEROXIDATION AND NITRITE LEVELS IN CCI RATS.

Treatment (mg/kg)	Nitrite (nmole/g tissue)	TBARS (nmole/g tissue)	SOD (% control)	Catalase (% control)
Sham	102.32 ± 7.86	59.34 ± 5.81	100 ± 4.56	100± 4.76
CCI	193. 35 ± 11.33[a]	168.21 ± 7.34[a]	47.12 ± 5.23[a]	51.34 ± 5.23[a]
CCI+ ACAM 100	174.42 ± 12.10	142.83± 8.02[b]	51.31 ± 5.12	55.67 ± 4.92
CCI + ACAM 200	144.28 ±8.32[b,c]	127.37 ± 6.32[b,c]	52.44 ± 6.21	57.64 ± 5.91
Pregabalin(30 mg/kg)	136.5 ± 9.45[b]	119.45 ± 6.13 [b]	55.23 ± 5.37	58.95 ± 4.76

Values are expressed as mean ± S.E.M. [a]P< 0.05 vs sham; [b]P < 0.05 vs CCI; [c]P < 0.05 vs CCI +PIO 10; [d]P < 0.05 vs CCI + FENO 100; Abbreviations: ACAM, acamprosate; CCI, chronic nerve constriction injury; TBARS, thiobarbituric acid reacting substances

TABLE 2: EFFECT OF ORAL ADMINISTRATION OF ACAMPROSATE (100 and 200 mg/kg) ON SOD, CATALASE, LIPID PEROXIDATION, AND NITRITE LEVELS IN SPINAL CORD OF CCI RATS.

Treatment(mg/ kg)	Nitrite(nmole/g tissue)	TBARS(nmole/g tissue)	SOD(% control)	Catalase(% control)
Sham	114..93 ± 18.23	49.54± 7.34	100 ± 5.32	100 ± 4.89
CCI control	421.46± 20.35[a]	146.76 ± 6.75[a]	54.45 ± 6.04[a]	55.65 ± 5.86[a]
CCI+ ACAM 100 mg	372.66 ± 19.13[b]	129.74± 6.62[b]	58.60±6.05[b]	59.44 ± 5.91[b]
CCI + ACAM 200 mg	315.73 ±17.54[b,c]	75.11 ± 5.30[b,c,e]	63.56 ± 7.66[b,c,e]	62.11 ± 6.76[b,c,e]
Pregabalin 30 mg	304.43 ± 18.84[b,d]	101.75 ± 7.22[b,d]	61.43 ± 6.71	63.21 ± 6.12

Values are expressed as mean ± S.E.M. [a]P< 0.05 vs sham; [b]P < 0.05 vs CCI; [c]P < 0.05 vs CCI +PIO 10; [d]P < 0.05 vs CCI + FENO 100; Abbreviations: ACAM, acamprosate; CCI, chronic nerve constriction injury; TBARS, thiobarbituric acid reacting substances.

Effect of repeated administration of acamprosate on locomotor activity:

All the animals had shown relatively similar motor activity score before surgery (i.e. on day 0). Further, the motor activity was normal in both sham-operated and CCI rats on day 7, 14 and 21 following surgery as compared to their respective scores observed on day 0. Chronic administration of acamprosate (100 and 200 mg/kg, p.o., for 2 weeks) do not produced any significant effect locomotor activity. Conversely, administration of pregabalin (30 mg/kg, i.p.) significantly decreased locomotor activity in CCI rats 2 h after its administration as compared to the vehicle treated CCI group as shown in **Table. 3**.

TABLE 3: EFFECT OF REPEATED ADMINISTRATION OF ACAMPROSATE ON LOCOMOTOR ACTIVITY (5MIN).

Treatment (mg/kg)	Day 0	Day 7	Day 14	Day 21
Sham	192 .5 ±10.4	187.8 ± 11.7	185.5	189.5
CCI	197.4 ± 9.5	191.8 ± 10.4	139.5 ± 7.2	190.3 ± 7.2
CCI +ACAM 100 mg	203.3 ± 12.4	200.6 ± 10.4	197.6 ±8.6	199.6 ± 7.5
CCI + ACAM 200 mg	193.5 ± 9.5	189.3 ± 7.4	192.6 ± 10.4	184.5 ± 6.6
CCI + Pregabalin 30 mg	201 ± 9.5	198.6 ± 9.2	171.5± 10.4 [a]	168.6 ± 8.8 [a]

Administration pharmacological agents were initiated on day 7 and continued once daily for 14 days. Values are mean ± S.E.M. [a]*P*< 0.05 vs CCI. Abbreviations: CCI, chronic nerve constriction injury; ACAM, acamprosate.

DISCUSSION: Although, carrageenan-induced paw edema is a most commonly used standard technique to screen anti-inflammatory activity, it can also be used as simple routine animal model for the evaluation inflammatory pain without any injury to the inflamed tissue [24, 30]. It is well recognized that nonsteroidal antiinflammatory drugs (NSAIDs) don not alter the nociceptive threshold in naïve animals, but do normalize the lowed nociceptive threshold induced by local inflammation after i.pl carrageenan administration [31, 32]. Thus, this method has been commonly for investigating or evaluating new drug therapies for inflammatory pain. Similarly, an injection of λ-

carrageenan (0.1 ml of 1% w/v) into i.pl. region of the hind paw has produced acute inflammation as evidenced by marked paw edema, and also mechanical hyperalgesia, which are persisted up to 24 hours post-carrageenan. It has been reported that thermal hyperalgesia associated with i.pl injection of carrageenan in rats is developed by 2hr and maintained up to 5 hr post-carrageenan, while, the developed thermal hyperalgesia was lasted 7 hr post-carrageeenan [33]. Therefore, in the present study was assessed on after administration of first dose of acamprosate (i.e., 2.30 and 4.30 h post-carrageenan).

In the present study, one hour pre-treatment with acamprosate (100 and 200 mg/kg, p.o) did not produce any effect on paw edema as well as thermal and mechanical hyperalgesia, as compared to vehicle treated carrageenan control. On the other hand, second dose acamprosate treatment at the 19 hr post-carrageenan significantly attenuated the mechanical hyperalgesia at a dose of 200 mg/kg, as compared to vehicle treated carrageenan control. After first dose oral administration of acamprosate at a dose of 200 mg/kg., but not 100 mg/kg significantly reversed the established mechanical hyperalgesia without producing significant effect on thermal hyperalgesia.

Pain is one of the classic signals of the inflammatory process. It is now accepted that the sensitization of primary sensory neurons is essential to inflammatory pain. In humans, this nociceptor sensitization usually leads to clinical conditions known as hyperalgesia (an increased response to a stimulus that is normally painful) or allodynia (pain due to a stimulus that does not normally provoke pain) [34]. Similarly, it has been demonstrated that inflammatory hypernociception characterized by mechanical allodynia and thermal hyperalgesia, in rats can induced by carrageenan, which is due to release of a cascade of mediators initiated by the production of the hypernociceptive cytokines TNF-α, interleukin-1β, and chemokines. These cytokines stimulate the release of the directly acting hypernociceptive mediators, particularly, prostaglandins, which directly act on nociceptive neurons [35, 36]. Nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit prostaglandin synthesis and prevent the development of hypernociception and thereby mainstay in the treatment of inflammatory pain [37]. The standardized extract extracts of Ginkgo biloba, EGb-761, which contains 24% of flavanoids, has shown to selectively reverse thermal hyperalgesia, but not mechanical allodynia in carrageenan induced inflammatory pain model. On the other hand, extract has not shown analgesic activity in normal animals [38]. Further, either systemic or local (i.pl) treatment with celecoxib, a selective COX-2 inhibitor, has shown to abolished inflammatory hyperalgesia and even produce hypoalgesia in carrageenan induced acute inflammatory pain in rats, without reducing oedema [39]. Moreover, enhanced formation of superoxide anion and peroxynitrite has been implicated in both carrageenan-induced paw edema and hyperalgesia[40, 41].

It is now clearly established that NP is associated with activation and/or modulation of multiple pathogenic mechanisms. Thus, there is an unmet need to characterize and develop newer agents, which are act at more than one site to effectively alleviate the symptoms and pathogenesis of NP. It has been demonstrated that acamprosate has similar effects, as compared to conventional anti-oxidants on oxygen free radicals during alcohol intoxication [42]. Radioligand binding studies suggested that acamprosate is act as a weak partial agonist effects on NMDA receptors through indirect actions on a polyamine site on the NMDAR complex [18, 19]. Other studies have suggested that acamprosate might interact with the polyamine site of the NMDA receptor [19, 20, 21].

In addition, there is some evidence that acamprosate might inhibit Ca^2 influx through NMDA and voltage-dependent Ca^2 channels [22]. It suggests that the observed anti-hyperalgesic activity selectively against mechanical hyperalgesia associated with carrageenan paw edema may be due to either inhibition of NMDA receptors or scavenging oxidative stress, but independent of anti-inflammatory activity.

The two week repeated administration of acamprosate (100 and 200mg/kg, p.o.) significantly reversed the established thermal and mechanical hyperalgesia and cold allodynia. These observed ameliorative effects on behavioral symptoms of

neuropathic pain are also associated with partial, but significant reduction of oxido-nitrosative stress markers. The functional N-methyl-D-aspartate receptor (NMDAR) is a heteromeric complex containing NR1 and NR2 subunits. The NR1 protein is an obligatory component for a functional NMDAR, with at least one of the NR2 subunit family member, of which NR2A and NR2B are the most abundant in adult rat dorsal horn (Nagy et al., 2004). Although the precise mechanism of chronic pain after spinal cord injury remains elusive, considerable evidence indicates that NMDAR in the superficial dorsal horn has been implicated as a major contributor to excitatory nociceptive transmission [12, 13]. Substantial evidence has established that activation of the NMDAR in the spinal dorsal horn is essential for central sensitization [14]. Various noncompetitive NMDAR antagonists (i.e., MK-801, ketamine, memantine and dextrorphan) decrease the development of allodynia and hyperalgesia following constrictive injury of the sciatic nerve and spinal nerve ligation [43, 44, 45, 46].

Moreover, intrathecal application of amino-5-phosphonopentanoate, a competitive NMDAR antagonist, reduces mechanical allodynia caused by spinal cord injury [47]. Systematic administration of the NR2B antagonist CP-101,606 or Ro 25-6981 decreases neuropathic pain in the sciatic nerve injury model with fewer side effects, as compared to other NMDAR antagonists [48]. Therefore, it has been suggested that NR2B at the spinal level does not appear to be important for the maintenance of neuropathic pain induced by nerve injury [14]. The reactive molecules such as •O2 -, •NO and ONOO- are shown to play an important role in the development and maintenance of NP [49]. In chronic constriction injury (CCI) model of rat neuropathic pain, heat hyperalgesia was reduced by systemically injected antioxidants [50, 51]. It suggests that the observed anti-hyperalgesic activity on repeated administration of acamprosate to either inhibition of NMDA receptors or inhibition of oxido-nitrosative stress in sciatic nerve injury-induced peripheral neuropathic pain.

SUMMARY AND CONCLUSION: The present study has been designed to investigate the effect of acamprosate in experimental models of peripheral neuropathic and inflammatory pain in Wistar rats. λ-carrageenan (0.1 ml of 1% w/v) into i.pl. region of the hind paw was injected to produce acute inflammation. Unilateral peripheral mono neuropathy was induced by sciatic nerve ligation. On the basis of results obtained in present study, the following salient findings may be summarized:

Both CCI of sciatic nerve and an i.pl injection of carrageenan are resulted in marked mechanical and thermal hyperalgesia. CCI-induced marked allodynia and hyperalgesia in ipsilateral paws, which is also associated with significantly elevated oxido-nitrosative stress in spinal cord and sciatic nerve.

The one hour pre-treatment with acamprosate (100 and 200mg/kg, p.o) did not produce any effect on paw edema as well as thermal and mechanical hyperalgesia, as compared to vehicle treated carrageenan control.

On the other hand, second dose acamprosate treatment at the 19hr post-carrageenan significantly attenuated the mechanical hyperalgesia at a dose of 200mg/kg, as compared to vehicle treated carrageenan control. After first dose oral administration of acamprosate at a dose of 200 mg/kg., but not 100mg/kg significantly reversed the established mechanical hyperalgesia without producing significant effect on thermal hyperalgesia.

The two week repeated administration of acamprosate (100 and 200mg/kg, p.o.) significantly reversed the established thermal and mechanical hyperalgesia and cold allodynia. These observed ameliorative effects on behavioral symptoms of neuropathic pain are also associated with partial, but significant reduction of oxido-nitrosative stress markers.

On the basis of above, it may be concluded that single dose treatment with acamprosate did not produce significant anti-inflammatory and anti-hyperalgesic effects, in carrageenan-induced inflammatory pain model. While, repeated administration of acamprosate ameliorates the behavioral symptoms of neuropathic pain observed CCI-induced NP. The observed effects may be

partly due to activation of anti-oxidant mechanisms, but independent of anti-inflammatory effects.

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How to cite this article:
Khanna G and Bijjem KR: Effect of Acamprosate in Experimental Models of Peripheral Neuropathic and Inflammatory Pain in Wistar Rats. Int J Pharm Sci Res 2015; 6(12): 5102-14.doi: 10.13040/IJPSR.0975-8232.6(12).5102-14.

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