



## Research Article

### Combined effect of baclofen and acamprosate in experimental models of peripheral neuropathic pain in Wistar rats

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## Abstract

**Objective:** Neuropathic pain (NP) is defined as pain associated with damage or permanent alteration of the peripheral or central nervous system. Current drug treatment for the management of neuropathic pain associated with various adverse effects. The present study was designed to investigate the combined effect of acamprosate and baclofen in experimental model of peripheral neuropathic pain in wistar rats. **Material and methods:** Neuropathic pain was induced by Chronic constriction injured (CCI) of sciatic nerve in rats. Acamprosate (100 and 200 mg/kg p.o) and baclofen (10 and 20 mg/kg p.o) was given in different groups for 14 days starting on 7th day post sciatic nerve ligation. Further combination of acamprosate(100 mg/kg p.o) and baclofen (10 mg/kg p.o) was also given to one group. On 1th, 3rd, 7th, 14th and 21st day behavioral parameters like mechanical allodynia and thermal hyperalgesia were assessed. Then animals were sacrificed on 22nd day and biochemical parameters (GSH, LPO, catalase, nitrite, SOD) were assessed. **Results:** Ligation of sciatic nerve significantly induced mechanical allodynia and thermal hyperalgesia with increase in oxidative stress (increase in LPO and nitrite) and decline of anti-oxidant enzyme levels (catalase, SOD, GSH) in sciatic nerve homogenate. Acamprosate (100 and 200 mg/kg p.o) and baclofen (10 and 20 mg/kg p.o) attenuated all the behavioural and biochemical parameters alone and/or combination. **Conclusion:** On the basis of obtained data, it can be concluded that acamprosate and baclofen alone and combination significantly prevented the development of NP in rats by exhibiting anti-hyperalgesic and anti-nociceptive effects, decreasing oxidative stress and increasing the anti-oxidant capacity.

**Keywords:** Baclofen, acamprosate, neuropathic pain, allodynia, hyperalgesia, oxidative stress

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## 1. Introduction

Neuropathic pain (NP) is defined 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]. 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 [3]. Thus, there is an unmet need to understand disease pathogenesis, identify and characterize novel targets, and develop newer agents which act at one or more sites in the pathogenesis of NP.

Various molecular modulators are associated with etiology of NP in which N-methyl-D-aspartate receptors (NMDAR), heteromeric complex containing NR1 and NR2 subunits, are documented

well for their contribution in NP [4]. 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 [5]. Various noncompetitive NMDAR antagonists (i.e., MK-801, ketamine, meantime and dextrorphan) decrease the development of allodynia and hyperalgesia following constrictive injury of the sciatic nerve and spinal nerve ligation [6]. Similarly, the  $\alpha\delta$  subunit specific binding inhibitors such as gabapentin and its analogue pregabalin have been shown to be effective in preclinical and clinical studies of neuropathic pain [7].

Gabapentin was first designed as a chemical analogue of  $\gamma$ -amino butyric acid, an inhibitory neurotransmitter, to treat spasticity and was later found to have anticonvulsant and antinociceptive activities in various seizure and pain models [8]. Recently, chronic intrathecal infusion of gabapentin was found to prevent nerve ligation-induced mechanical allodynia and thermal hyperalgesia without causing obvious neuropathological changes in spinal cord and cauda equine [9]. In continuation to associated factors in NP, both oxidative & nitrosative stress are major contributor in its pathology. In pathological conditions, intracellular ROS level is elevated due to increased production or impaired removal. In chronic constriction injury (CCI) model of rat neuropathic pain, heat hyperalgesia was reduced by systemically injected antioxidants [10].

It has been suggested that a combination drug treatment strategy, wherein several pain-related mechanisms are simultaneously engaged, could be more efficacious than treatment against individual mechanisms alone. In the present study, effect of baclofen is checked in combination with acamprosate for the attenuation of NP and their synergistic anti-nociceptive action in experimental peripheral neuropathic pain in Wistar Rats. Baclofen, an agonist for the GABA<sub>B</sub> receptors, has performed well in both preclinical and clinical studies of NP and nociceptive pain. Moreover it has been used successfully for complex regional pain syndrome (CRPS). Recently in a study by Saulino, (2014) has observed the positive effects of baclofen in spinal cord injury (SCI) associated pain.

Therefore, the present study has been designed to investigate the synergistic effect of baclofen &

acamprosate in experimental models of peripheral neuropathic and inflammatory pain in wistar rats.

### Experimental animals

Wistar rats either sex, weighing 170-250 gm, were used in present study. Animals were obtained from Central Animal House of ISF College of Pharmacy, Moga, Punjab (India). The animals were kept in polyacrylic cages and maintained under standard housing conditions (room temperature  $22\pm 20^\circ\text{C}$  and relative humidity of 60-65%) with 12h light/dark reverse cycle. The food in the form of dry pallets and water were made available *ad libitum*. All behavioral experiments were carried out between 9:00 to 15:00 hrs. The protocol was reviewed and approved by the Institutional Animal Ethics Committee with approval number ISFCP/CPCSEA/M9/221 dated 18<sup>th</sup> January, 2014 and the animal experiments were carried out in accordance with the Indian National Science Academy Guidelines for use and care of animals.

### Drugs and chemicals

Drugs like acamprosate and baclofen was obtained from Sigma Aldrich India. acamprosate and baclofen were freshly prepared by solubilising in 0.5% of carboxy methyl cellulose (CMC). Unless stated, all other chemicals and biochemical reagents of highest analytical grade quality were used.

### Induction of Chronic Constrictive Injury of Sciatic Nerve Ligation

The mononeuropathy was produced according to the method described by Bennett and Xie (1988). 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 1 mm interval. Great care was taken not to interrupt epineural blood flow during tying the ligature. After surgery, all animals received gentamycin (5 mg/kg, i.p.) to prevent sepsis.

### Treatment schedule CCI model

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 200mg/kg) and baclofen (10 and 20 mg/kg) in CCI model treatment was started

on day 7 after nerve injury and was continued upto next 14 days.

### Table 2: Experimental Protocol

Animals were randomly divided into six groups (n=6)

<b>Group 1</b>	Normal Control
<b>Group 2</b>	CCI (Disease control)
<b>Group 3</b>	CCI + Baclofen (10mg/kg)
<b>Group 4</b>	CCI + Baclofen (20mg/kg)
<b>Group 5</b>	CCI + Acamprosate (100mg/kg)
<b>Group 6</b>	CCI + Acamprosate (200mg/kg)
<b>Group 7</b>	CCI + Acamprosate (100 mg/kg) + Baclofen (10 mg/kg)

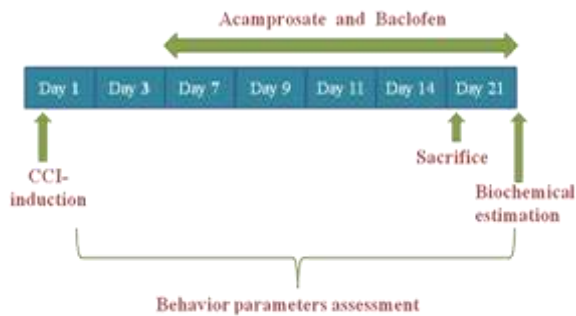


Fig. 3 Experimental procedure and treatment schedule

### Assessment of Behavioral parameters

#### Assessment of Mechanical allodynia in rats (Von Frey test)

The mechanical threshold for touch sensitivity was measured in hind paw, using an automated apparatus for applying device consisting of a steel rod against the plantar region of the paw by increasing force until the animal withdraw its paw. It is done using Dynamic plantar Aesthesiometer 37400-002; UgoBasile, Comerio, Italy. The maximum force was set at 50 gm prevent tissue damage, and the ramp speed was 0.5 g/s with cut-off is set to 20 seconds. The paw withdrawn latency is checked [11].

#### Assessment of Thermal hyperalgesia in rats

Hyperalgesia to thermal stimulation was determined using a Plantar Test Apparatus (37370-002 UgoBasile, Comerio, Italy) modelled as described by Hargreaves et al. 1988. Rats were placed individually in Plexiglas cubicles mounted on a glass surface maintained at  $25 \pm 2^\circ\text{C}$ . 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 right hind paw, and paw withdrawal latencies (PWLs) were recorded. A cut-off latency of 20s was imposed to avoid tissue damage [12].

### 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 ( $4-6^\circ\text{C}$ ) for a period of 15secs (cut off time) [13]. 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.

### Assessment of Biochemical parameters

#### Tissue preparation

In this study, at the end of treatment schedule on day 21, 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 15 min at 2000g to obtain the clear supernatant for the estimation of oxidative stress markers.

#### Measurement of lipid peroxidation

The quantitative measurement of lipid peroxidation in liver was performed according to the method of Will's, 1965. The amount of malondialdehyde (MDA), a measure of lipid peroxidation was assayed in the form of thiobarbituric acid reacting substances (TBARS). TBARS were quantified using an extinction coefficient of  $1.56 \times 10^5 \text{ M}^{-1}\text{cm}^{-1}$  and expressed as n mol of malondialdehyde per mg protein.

#### Estimation of Reduced Glutathione

The quantitative measurement of Glutathione (GSH) in liver was performed according to the method of Ellman, 1959. Glutathione protects cells from the free radicals produced through oxidation. The ratio of reduced GSH to oxidized GSH within the cells can be used to measure cellular toxicity. In healthy cells, 90 percent of the GSH should be in its reduced form [14].

### Estimation of Nitrite

The accumulation of nitrite in the supernatant, an indicator of the production of nitric oxide (NO), was determined with a colorimetric assay with Greiss reagent, 1% sulfanilamide and 2.5% phosphoric acid] as described by Green [15]. Spectrophotometer, Shimadzu, Japan. The concentration of nitrite in the supernatant was determined from a sodium nitrite standard curve and expressed as percentage of control.

### Estimation of Catalase

Catalase activity was assayed by the method of Luck (1971), wherein breakdown of hydrogen peroxides (H<sub>2</sub>O<sub>2</sub>) is measured at 240 nm. The results were expressed as micromole H<sub>2</sub>O<sub>2</sub> decomposed per milligram of protein/min.

### Estimation of Protein

Protein estimation was estimated by Biuret method [16] using bovine serum albumin (BSA) as standard.

### Estimation of Superoxide dismutase activity

Superoxide dismutase (SOD) activity was assayed by the method of Kono, wherein the reduction of nitrazo blue tetrazolium (NBT) was inhibited by the superoxide dismutase and is measured. The assay system consists of EDTA 0.1 mM sodium carbonate 50 mM and 96 mM of nitro blue tetrazolium (NBT). In the cuvette, 2ml of the above mixture, 0.05 ml of hydroxylamine and 0.05 ml of the supernatant was added and auto-oxidation of hydroxylamine was measured for 2 min at 30 sec interval by measuring absorbance at 560nm using Perkin Elmer Lambda 20 spectrophotometer.

### Statistical analysis

The results are expressed as mean  $\pm$  SEM. The behavioral data of Morris were analyzed by two ways analysis of variance (ANOVA) followed by Bonferroni's post hoc test for multiple comparisons whereas the biochemical data were analyzed by one way ANOVA followed by Bonferroni post hoc test. In all tests, values with  $p < 0.05$  was considered to be statistically significant.

### Results

#### Behavioral parameters:

#### Effect of Baclofen and Acamprosate on CCI-induced mechanical hyperalgesia in rats

Sciatic nerve ligation significantly produced hyperalgesia and the pain threshold was decreased on day 3<sup>rd</sup>, 7<sup>th</sup>, 14<sup>th</sup> and 21<sup>st</sup> in CCI-

control group as compared to vehicle treated group. Pretreatment with baclofen (10 and 20 mg/kg *p.o*) and acamprosate (100 and 200 mg/kg *p.o*) started on 7<sup>th</sup> day significantly increased nociceptive threshold for mechanical hyperalgesia on day 21 as compared to the CCI control group. Further, low dose combination of baclofen (10 mg/kg, *p.o*) and acamprosate (100 mg/kg, *p.o*) showed synergistic effect as compared to their effect alone and CCI control group.

#### Effect of Baclofen and Acamprosate on CCI-induced thermal hyperalgesia in rats

Sciatic nerve ligation significant produced development of thermal hyperalgesia, indicated by decrease in paw withdrawal threshold on day 3<sup>rd</sup>, 7<sup>th</sup>, 14<sup>th</sup> and 21<sup>st</sup> in CCI-control rats as compared to vehicle treated group. Pretreatment with baclofen (10 and 20 mg/kg *p.o*) and acamprosate (100 and 200 mg/kg *p.o*) started on 7<sup>th</sup> day significantly increased nociceptive threshold for thermal hyperalgesia on day 21 as compared to the CCI control group. Further, low dose combination of baclofen (10 mg/kg, *p.o*) and acamprosate (100 mg/kg, *p.o*) showed synergistic effect as compared to their effect alone and CCI control group.

#### Effect of Baclofen and Acamprosate on CCI-induced cold allodynia in rats

Sciatic nerve ligation significant produced development of cold allodynia, indicated by decrease in paw withdrawal threshold on day 3<sup>rd</sup>, 7<sup>th</sup>, 14<sup>th</sup> and 21<sup>st</sup> in CCI-control rats as compared to vehicle treated group. Pretreatment with baclofen (10 and 20 mg/kg *p.o*) and acamprosate (100 and 200 mg/kg *p.o*) started on 7<sup>th</sup> day significantly increased nociceptive threshold for cold allodynia on day 21 as compared to the CCI control group. Further, low dose combination of baclofen (10 mg/kg, *p.o*) and acamprosate (100 mg/kg, *p.o*) showed synergistic effect as compared to their effect alone and CCI control group.

#### Biochemical parameters

#### Effect of Baclofen and Acamprosate on LPO (TBARS) level in CCI-induced neuropathic pain in rats

Chronic constrictive injury of sciatic nerve ligation in rats resulted in significant increase in the levels of TBARS as compared to vehicle treated group. Pretreatment with baclofen (10 and 20 mg/kg *p.o*) and acamprosate (100 and 200 mg/kg *p.o*) started on 7<sup>th</sup> day significantly

decreased TBARS level in rats on 22<sup>nd</sup> day. Further, low dose combination of baclofen (10 mg/kg, p.o) and acamprosate (100 mg/kg, p.o) showed synergistic effect as compared to their effect alone and CCI control group.

#### **Effect of Baclofen and Acamprosate on Nitrite level in CCI-induced neuropathic pain in rats**

Chronic constrictive injury of sciatic nerve ligation in rats resulted in significant increase in the nitrite levels as compared to vehicle treated group. Pretreatment with baclofen (10 and 20 mg/kg p.o) and acamprosate (100 and 200 mg/kg p.o) started on 7<sup>th</sup> day significantly decreased nitrite level in rats on 22<sup>nd</sup> day. Further, low dose combination of baclofen (10 mg/kg, p.o) and acamprosate (100 mg/kg, p.o) showed synergistic effect as compared to their effect alone and CCI control group.

#### **Effect of Baclofen and Acamprosate on reduced GSH level in CCI-induced neuropathic pain in rats**

Chronic constrictive injury of sciatic nerve in rats significantly decreased the level of reduced glutathione in rats and further pretreatment with baclofen (10 and 20 mg/kg p.o) and acamprosate (100 and 200 mg/kg p.o) started on 7<sup>th</sup> day significantly increased reduced GSH level in rats on 22<sup>nd</sup> day. Further, low dose combination of baclofen (10 mg/kg, p.o) and acamprosate (100 mg/kg, p.o) showed synergistic effect as compared to their effect alone and CCI control group.

#### **Effect of Baclofen and Acamprosate on SOD level in CCI-induced neuropathic pain in rats**

Chronic constrictive injury of sciatic nerve in rats significantly decreased the level of SOD in rats and pretreatment with baclofen (10 and 20 mg/kg p.o) and acamprosate (100 and 200 mg/kg p.o) started on 7<sup>th</sup> day significantly increased SOD level in rats on 22<sup>nd</sup> day. Further, low dose combination of baclofen (10 mg/kg, p.o) and acamprosate (100 mg/kg, p.o) showed synergistic effect as compared to their effect alone and CCI control group.

#### **Effect of Baclofen and Acamprosate on catalase level in CCI-induced neuropathic pain in rats**

Chronic constrictive injury of sciatic nerve in rats significantly decreased the level of catalase in rats and further pretreatment with baclofen (10 and 20 mg/kg p.o) and acamprosate (100 and 200

mg/kg p.o) started on 7<sup>th</sup> day significantly increased catalase level in rats on 22<sup>nd</sup> day. Further, low dose combination of baclofen (10 mg/kg, p.o) and acamprosate (100 mg/kg, p.o) showed synergistic effect as compared to their effect alone and CCI control group.

#### **Discussion**

NP is a chronic debilitating condition characterized by hyperalgesia and allodynia. A lot of effort has been put in the establishment of new and effective therapies but treatment of NP is still a challenging issue as the whole pain processing is altered. The available drugs for NP include anti-depressants, anticonvulsants, sodium and calcium channel blockers, N-methyl-D-aspartic acid (NMDA) receptors antagonists and Opioids. These drugs provide a transient relief from NP and produce severe CNS related, dose-limiting side effects. Thus, there is an unmet need to understand disease pathophysiology, identify and characterize novel targets, and develops newer agents which act at one or more sites in the pathophysiology of NP.

The abnormal activation of inflammatory and immune system, particularly glial activation and subsequent release of pro-inflammatory cytokines, in the peripheral and central nervous system play a substantial role in the development of NP. In addition, oxidative-stress has also been shown to play an important pathogenic role in the peripheral as well as central sensitization and subsequent development of NP. Moreover, selective inhibition of activation and/ or recruitment of inflammatory cells, pharmacological modulation of oxidative stress and expression of pro-inflammatory cytokines ameliorate hypersensitivity, following nerve injury.

The Chronic constriction injury (CCI) of sciatic nerve produces robust mechanical hyperalgesia, thermal hyperalgesia and cold allodynia in the ipsilateral paw started within 3–5 days of the post-nerve injury and that lasts for about 40–50 days before progressing into hyperalgesia. Similarly, in the present study, ipsilateral PWLs in CCI-control rats showed marked thermal hyperalgesia, mechanical hyperalgesia and cold allodynia on day 3 and reached steady state between day 7 and day 21 post-sciatic nerve ligation. Also, CCI-control group animals showed significant increase in levels of pro-inflammatory cytokines and oxido-nitrosative stress, along with significant decrease in antioxidant enzyme levels.

The observed effects are in line with the earlier reports. Two week administration of low and high dose of both acamprosate (100 and 200 mg/kg *p.o*) and baclofen (10 and 20 mg/kg *p.o*) starting from day 7 after surgery significantly increased the pain perception and the antioxidant enzyme levels and decreased oxidative stress on day 22 in sciatic nerve tissue homogenate. Further, combined treatment with low dose of acamprosate(100 mg/kg *p.o*) and baclofen (10 mg/kg *p.o*) significantly decreased NP as assessed by decreased thermal hyperalgesia, mechanical hyperalgesia, cold allodynia and oxidative stress (LPO, nitrite) and increased antioxidant enzyme level (GSH, catalase, SOD) measured on day 22 in sciatic nerve tissue homogenate.

Accumulating clinical and pre-clinical evidences clearly suggests that increased generation of advanced glycation end-products [17], mitochondrial dysfunction [18], activation of nuclear factor- $\kappa$ B, and subsequent generation of pro-inflammatory cytokines are involved in the development of NP. Moreover, recent studies implicate infiltration and activation of inflammatory cells[19], and subsequent release of pro-inflammatory cytokines such as interleukin-1 $\beta$ , and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) has been shown to cause peripheral neuronal hypersensitization as well as neuronal demyelination process. Indeed, activated microglial cells in the CNS are an important source of release of a variety of neuromodulators and neuroactive substances like reactive oxygen species (ROS) [20], glutamate [21], Nitric oxide (NO) and peroxynitrite, prostaglandins [22], and pro-inflammatory cytokines (IL-1 $\beta$  and TNF- $\alpha$ ) [23], are all implicated directly in the development of NP.

The inflammatory and immune mechanisms in the peripheral and central nervous system play an important role in the development and maintenance of peripheral neuropathic pain. Further, both the glia and neurons express receptors for various neurotransmitters and neuromodulators involved in central sensitization [24]. Glial cells, such as microglia and astrocytes, are activated in response to nervous system damage with the upregulation of cell surface markers, including macrophage antigen complex-1, toll like receptor-4, glial fibrillary acidic protein and subsequent release of several inflammatory mediators. Pro-inflammatory cytokines, such as TNF- $\alpha$ , IL-1 $\beta$ , and IL-6, play a critical role in the development of central sensitization and release

of nociceptive mediators such as prostaglandins (PG), substance P and NO. Elevated levels of IL-1 $\beta$ , TNF- $\alpha$  and IL-6 and other cytokines suggest a profound inflammatory cascade after peripheral nerve injury, which may contribute to central sensitization through direct actions on neuronal receptors and indirect actions upon glial cells.

Consistent with these reports, in the present study, the vehicle treated CCI-control rats developed a significant increase in inflammatory and oxido-nitrosative stress, along with significant decrease in anti-oxidant levels. This indicates that these pathogenic mechanisms play an important role in the development and maintenance of NP. However, concurrent administration of acamprosate and baclofen significantly attenuated the oxido-nitrosative stress and pro-inflammatory cytokines in the sciatic nerve which are involved in NP and thus GSPE reduced the thermal and mechanical hyperalgesia and allodynia by reducing the level of these pro-inflammatory cytokines and inhibiting free radical generation and reducing the expression of iNOS. Therefore, these data and results in the present study, suggests that acamprosate and baclofen are effective in treating the neuropathic pain through anti-oxidant and anti-inflammatory mechanisms and thus producing anti-hyperalgesic and anti-nociceptive effects.

Chronic treatment with acamprosate has shown to reduce the anti-inflammatory and anti-oxidant effects. Acamprosate has shown to inhibit the release of various excitatory cytokines (substance P, CGRP, cytokines) which cause NP and exhibits reduction in the expression of various soluble adhesion molecules, ICAM-1, VCAM-1 and E-selectin in plasma of autoimmune disease patients. It also decline the protein expression of p65 and subsequent suppression of proinflammatory factors i.e. TNF- $\alpha$ , IL-17, IL-1 $\beta$ , prostaglandins,  $\beta$ -NAG (N-Acetyl- $\beta$ -D-glucosaminidase) thereby indicating the potent anti-inflammatory effect. Acamprosate has been reported to produce excitatory or inhibitory effects on NMDA receptors depending on its concentration and receptor activity [25] showed that acamprosate inhibits glutamate induced calcium entry in cultures of neocortical neurons without affecting glutamate neurotoxicity. Therefore, the possible mechanism for beneficial effect of acamprosate in NP might be NMDA antagonism, anti-inflammatory as well as antioxidant action.

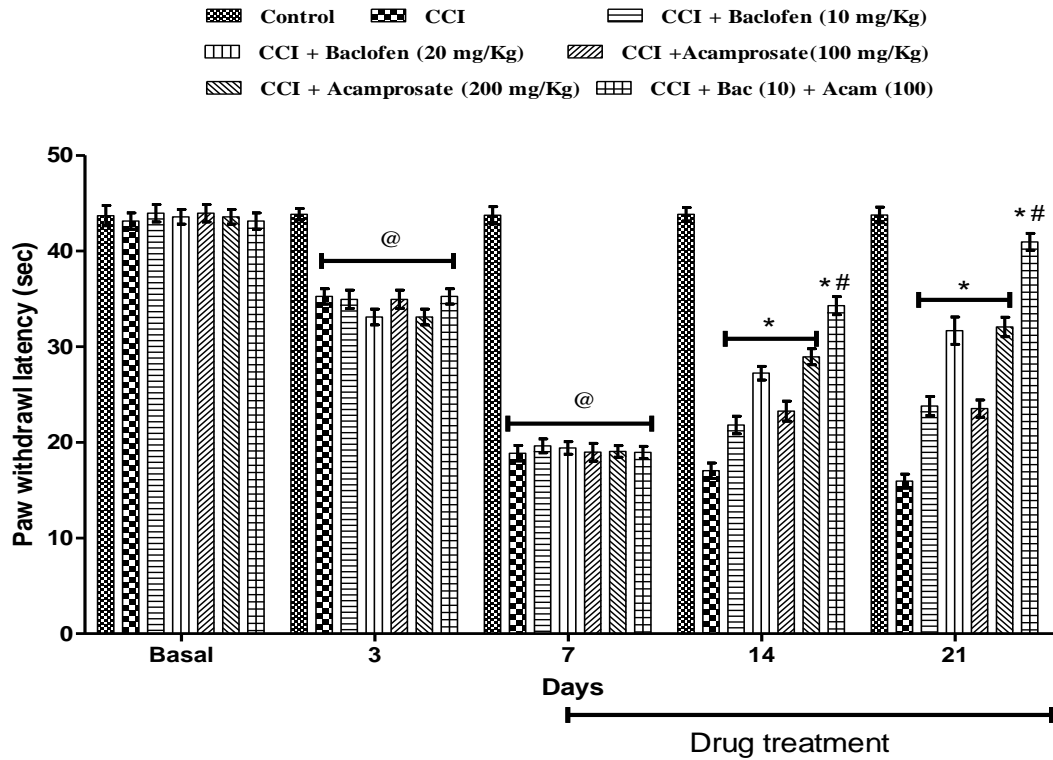


Fig 1: Effect of Baclofen and Acamprosate on CCI-induced mechanical hyperalgesia in rats: Data are expressed in mean  $\pm$  SEM @P<0.05 versus vehicle treated, \*P<0.05 versus CCI-Control, #P<0.05

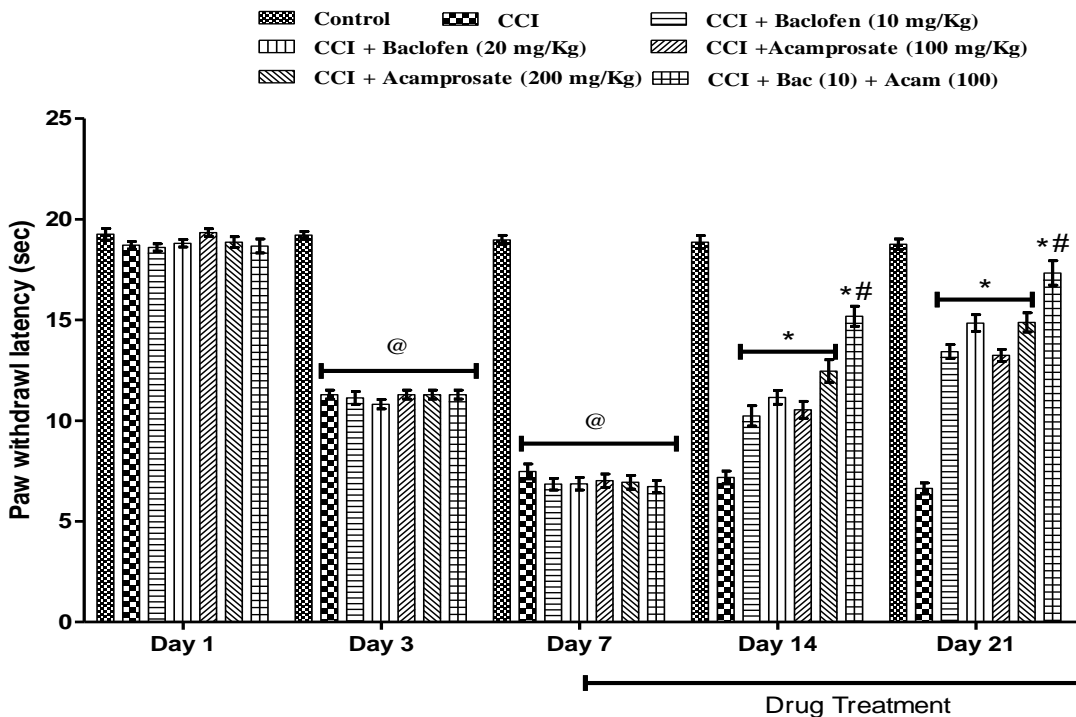
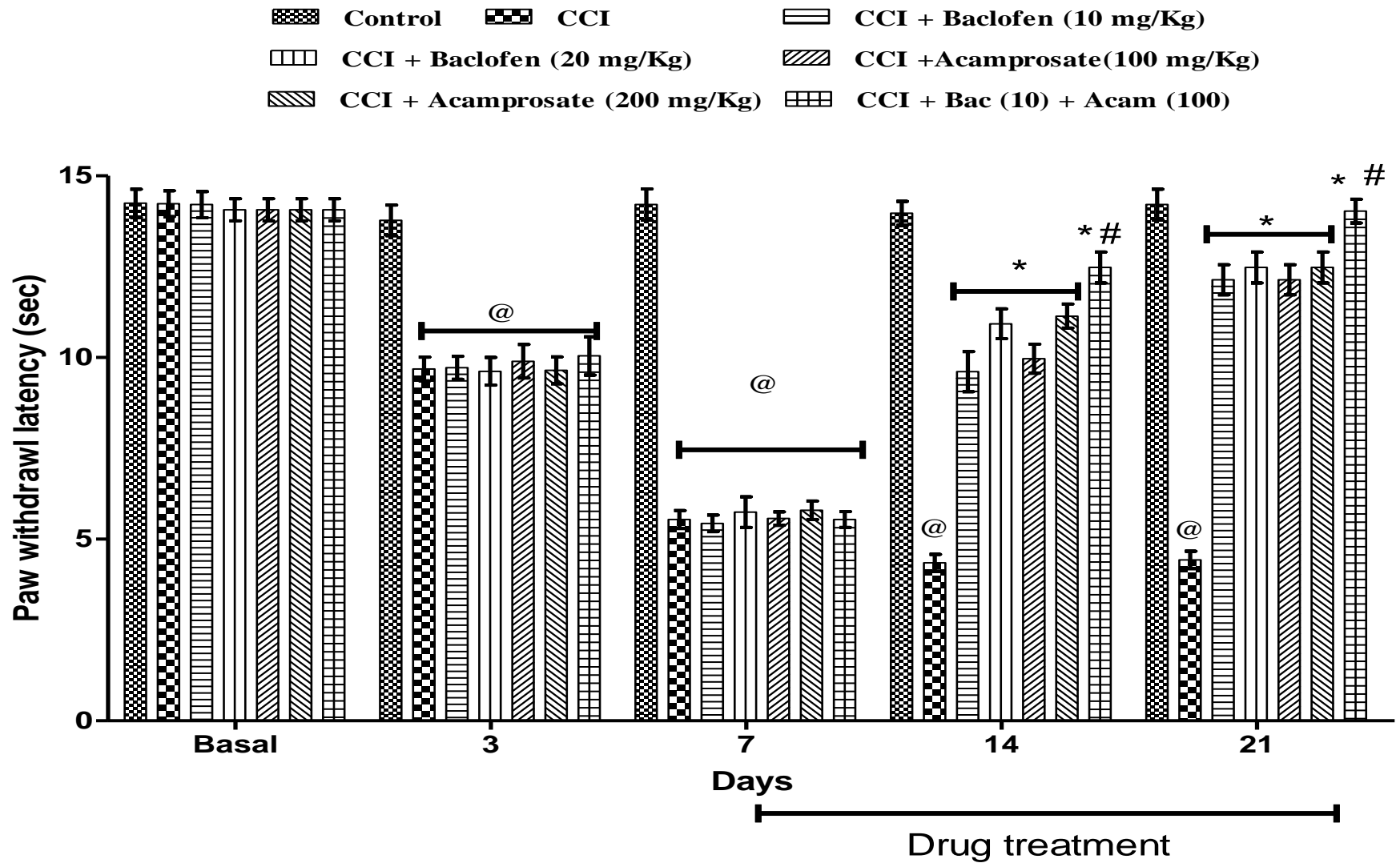
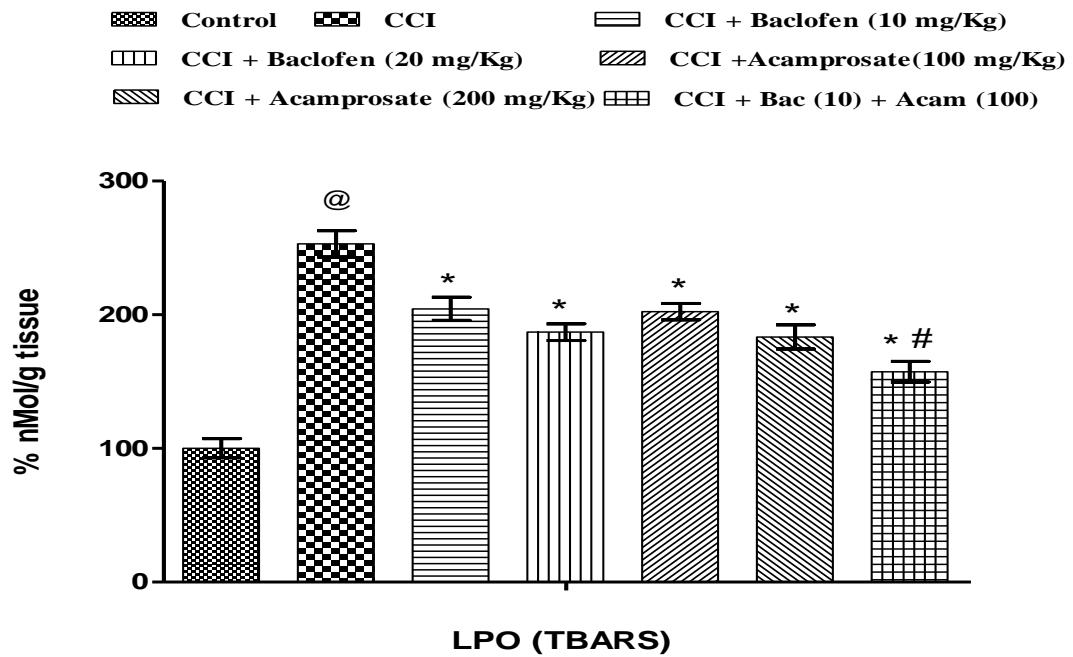


Fig 2: Effect of Baclofen and Acamprosate on CCI-induced thermal hyperalgesia in rats: Data are expressed in mean  $\pm$  SEM @P<0.05 versus vehicle treated, \*P<0.05 versus CCI-Control, #P<0.05 [Baclofen (10 mg/kg p.o alone and Acamprosate (100 mg/kg p.o alone)] treated group on 21<sup>st</sup> day.

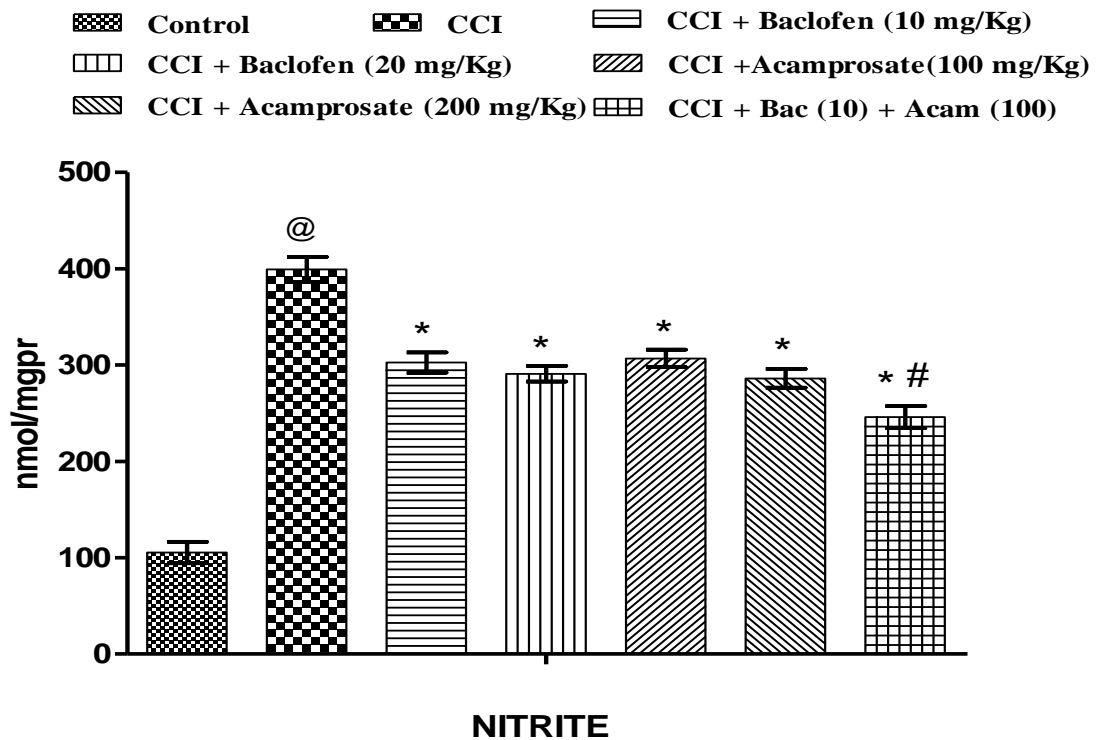


**Fig 3: Effect of Baclofen and Acamprosate on CCI-induced cold allodynia in rats:** Data are expressed in mean  $\pm$  SEM @P<0.05 versus vehicle treated, \*P<0.05 versus CCI-Control, #P<0.05 [Baclofen (10 mg/kg p.o alone and Acamprosate (100 mg/kg p.o alone)] treated group on 21<sup>st</sup> day.

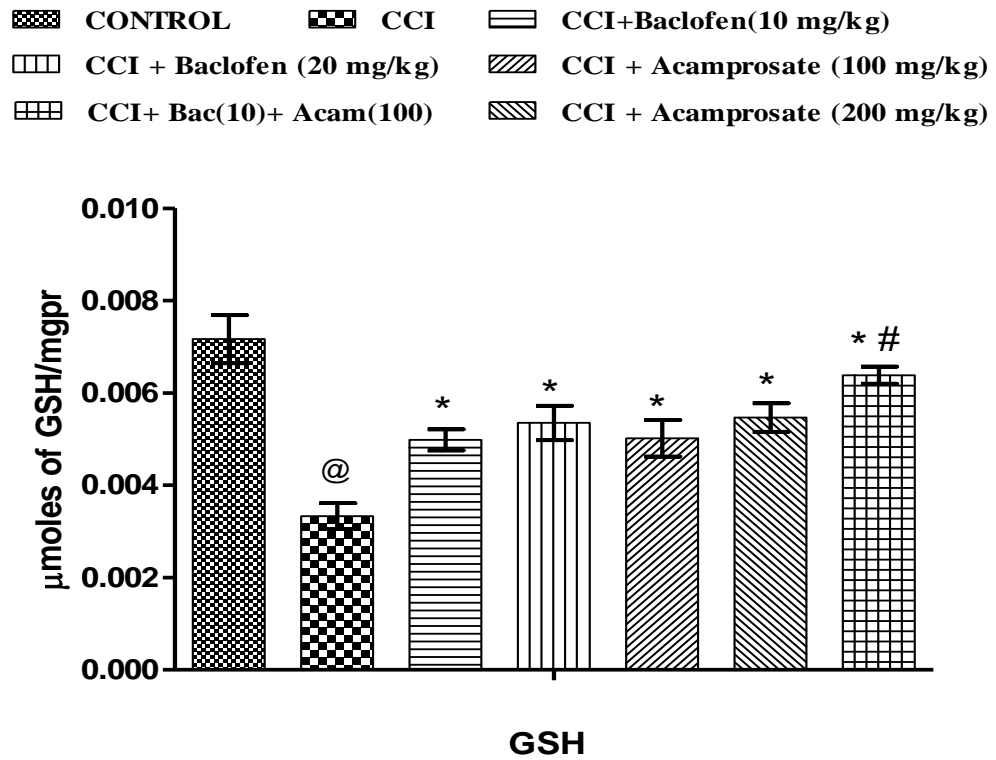




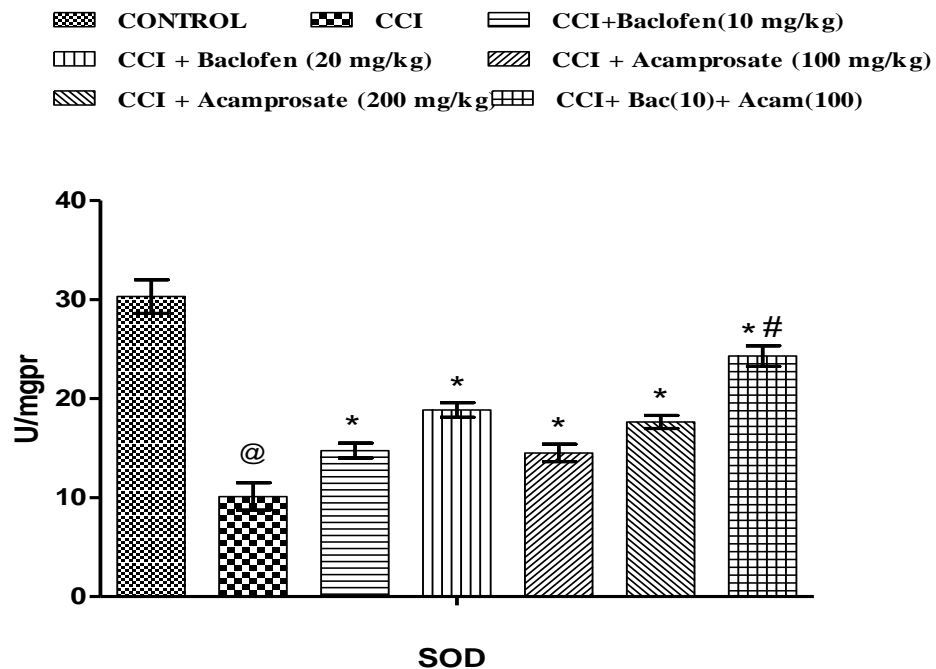
**Fig 4: Effect of Baclofen and Acamprosate on LPO (TBARS) level in CCI-induced neuropathic pain in rats:** Data are expressed in mean  $\pm$  SEM @P<0.05 versus vehicle treated, \*P<0.05 versus CCI-Control, #P<0.05 [Baclofen (10 mg/kg p.o alone and Acamprosate (100 mg/kg p.o alone)] treated group on 21<sup>st</sup> day.



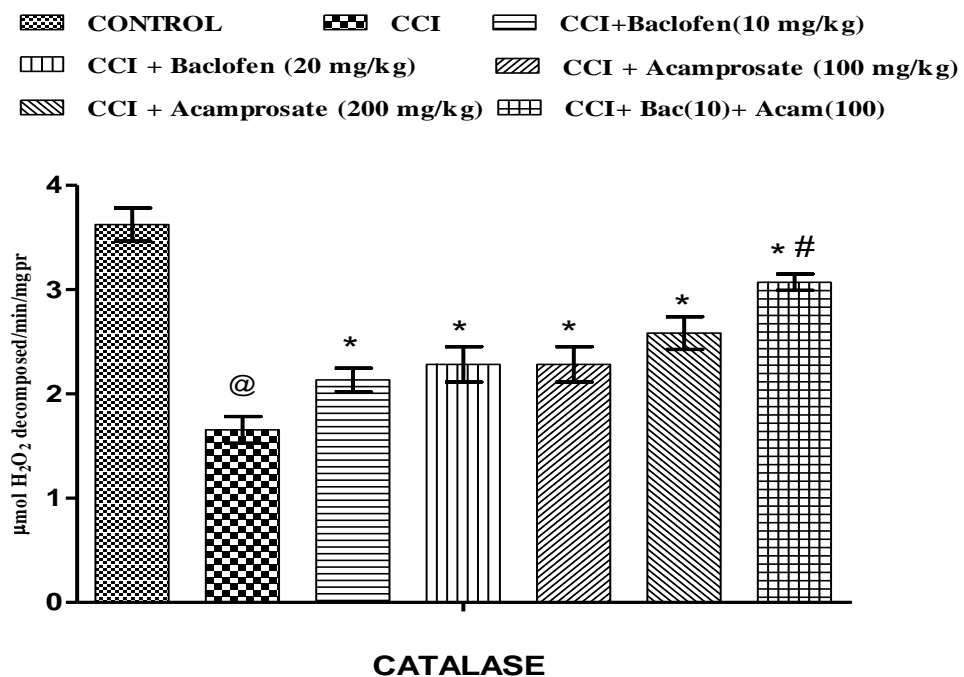
**Fig 5: Effect of Baclofen and Acamprosate on Nitrite level in CCI-induced neuropathic pain in rats:** Data are expressed in mean  $\pm$  SEM @P<0.05 versus vehicle treated, \*P<0.05 versus CCI-Control, #P<0.05 [Baclofen (10 mg/kg p.o alone and Acamprosate (100 mg/kg p.o alone)] treated group on 21<sup>st</sup> day.



**Fig 6: Effect of Baclofen and Acamprosate on reduced GSH level in CCI-induced neuropathic pain in rats:** Data are expressed in mean  $\pm$  SEM @P<0.05 versus vehicle treated, \*P<0.05 versus CCI-Control, #P<0.05 [Baclofen (10 mg/kg p.o alone and Acamprosate (100 mg/kg p.o alone)] treated group on 21<sup>st</sup> day.



**Fig 7: Effect of Baclofen and Acamprosate on SOD level in CCI-induced neuropathic pain in rats:** Data are expressed in mean  $\pm$  SEM @P<0.05 versus vehicle treated, \*P<0.05 versus CCI-Control, #P<0.05 [Baclofen (10 mg/kg p.o alone and Acamprosate (100 mg/kg p.o alone)] treated group on 21<sup>st</sup> day.



**Fig 8: Effect of Baclofen and Acamprosate on catalase level in CCI-induced neuropathic pain in rats:** Data are expressed in mean  $\pm$  SEM @P<0.05 versus vehicle treated, \*P<0.05 versus CCI-Control, #P<0.05 [Baclofen (10 mg/kg p.o alone and Acamprosate (100 mg/kg p.o alone)] treated group on 21<sup>st</sup> day.

The discovery of the exogenous GABAB receptor ligand, baclofen, i.e., p-chlorophenyl-GABA, was a milestone in the characterization of these receptors. Baclofen was synthesized by Heinrich Keberle in 1962, 30 years before a GABAB receptor was cloned [26]. Baclofen is a lipophilic GABA derivative and possesses a high affinity and strong intrinsic activity for GABAB receptors. Baclofen is an optically active compound, and its R isomer shows a three times greater affinity/efficacy for GABAB receptors than the racemate. Structure-activity studies have led to the discovery of 3-(4-pyridyl) methyl ether derivative '9d' (derivative of baclofen) which has 25- to 50-fold greater functional potency than R-baclofen at human and rodent GABAB receptors in vitro and easily crosses blood-brain barrier following systemic administration.

Baclofen (p-chloro- $\beta$ - phenyl-GABA) is a selective GABA B agonist and is demonstrated to produce catatonic, antidepressant and analgesic effects. Preclinical studies have evidenced that GABAergic drugs produce analgesia. Baclofen is clinically effective in the treatment of spasticity of various origins. The muscle-relaxant action most likely reflects a reduction in spinal reflex excitability, as suggested by Baclofen's capacity to suppress mono and polysynaptic reflexes, excitatory

postsynaptic potentials, and dorsal root potentials [27]. Baclofen has also been reported to suppress pain in patients suffering from spastic conditions. But this apparent analgesia may have been secondary to muscle relaxation.

Various mechanisms have been proposed to be involved in abnormal pain behavior and analgesic tolerance, including activation of NMDA and MAPK receptor, PKA, PKC, receptor desensitization, an increased oxidative stress, increase in cytokines and NO levels. Accumulating evidences indicates that both agents are coupled to Gi/Go GTP-binding proteins that inhibit adenylcyclase activity, block voltage-dependent calcium channels, activate potassium channels and stimulate the MAP kinase cascade.

Thus in the present study, we conclude that the low and high dose of baclofen and acamprosate significantly attenuated the pain threshold induced hyperalgesia and allodynia and increases the level of various anti-oxidants and also the combination of low dose of baclofen and acamprosate produced synergistic effect. Therefore, baclofen and acamprosate can be used as an adjuvant therapy with available drugs for the clinical implications.

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