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Research Article

Anti-nociceptive effects of taurine and caffeine in sciatic nerve ligated wistar rats: involvement of autonomic receptors

W. Abdulmajeed and Owoyele B.V.*

Department of Physiology, Faculty of Basic Medical Sciences, University of Ilorin, Ilorin, Nigeria.

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ABSTRACT

In this study, we investigated the effects of co-administration of taurine and caffeine on thermally induced pain in sciatic nerve ligated rats as well as the roles of autonomic receptors. Rats were rendered neuropathic by unilateral sciatic nerve ligation. The anti-hyperalgesic effect of combined systemic (i.p.) administration of taurine and caffeine were assessed using tail flick tests and hot plate test for two weeks. To determine the involvement of autonomic nervous system, the study examined how administration of cholinergic (atropine and hexamethonium) and adrenergic (prazosin and propranolol) receptor blockers altered the combined effect of taurine and caffeine. Likewise, the serum level of oxidative stress marker malondialdehyde (MDA) was evaluated. The results showed that co-administration of taurine and caffeine attenuated thermal hyperalgesia in sciatic nerve ligated rats as shown by significant ($p<0.05$) increase in tail and paw withdrawal latencies in the treated groups compared to the ligated control group after two weeks of administration. The anti nociceptive effects were reversed by pre-treatment with cholinergic blockers especially atropine while the adrenergic blockers spared the effects of taurine and caffeine. Also, the increase in tissue level of MDA induced by sciatic nerve ligation was significantly attenuated by combined administration of high dose of taurine and caffeine. It can be concluded that co-administration of taurine and caffeine attenuates thermal hyperalgesia in sciatic nerve-ligated rats (a model of neuropathic pain) and this effect involves cholinergic system. The findings suggest that co-administration of taurine and caffeine might be useful for the treatment of neuropathic pain.

INTRODUCTION

Neuropathic pain has been defined as pain caused by a lesion of the peripheral or central nervous system (or both), manifesting with sensory symptoms and signs (positive and negative sensory phenomena (Backonja, 2003). Neuropathic pain is often associated with the appearance of abnormal sensory signs, such as allodynia (pain as a result of a stimulus which does not normally provoke pain) or hyperalgesia (an increased response to a stimulus which is normally painful) and often reported as having a lancinating or continuous burning character (Bridges *et al.,* 2001). This form of pain requires more research and application of novel therapeutic drugs (Shmader, 2002). Clinically, tricyclic anti-depressants and some anticonvulsants such as gabapentin have been employed in the management of neuropathic pain (Sindrup and Jensen, 2002).

Taurine (2-aminoethanesulfonicacid) is an organic osmolyte (Terada *et al.,* 2011) that is not incorporated into proteins. In the central nervous system, taurine has been implicated in cell volume regulation, neuroprotection and inhibitory neurotransmission (Gupta, 2006). Taurine is an important trophic factor in the development of the central nervous system (CNS) (Menzie, 2012). Antinocicepive effects of taurine in acute and inflammatory pain models have been documented (Silva *et al.,* 1993; Serrano *et al.,*1994) and its depletion has also been implicated in the development of neuropathic pain (Hansen, 2001). Also, Li *et al*., (2005) showed that dietary supplementation with taurine suppressed hyperalgesia in streptozotocin (STZ)-induced diabetic rats, likewise taurine induced attenuation of autotomic behaviour in genetically

*Address for correspondence:
Email: deleyele@yahoo.com or
owoyele@unilorin.edu.ng

selected Sabra strain rats has been reported (Belfer *et al.,*1998).

Caffeine is widely consumed for its central nervous system (CNS) stimulant effects which include alertness and decreased fatigue. It is present in a variety of beverages (coffee, tea, energy drinks) and some foods (chocolate, desserts); it is also available as a drug where it is used as a stimulant, or is added to analgesics in over the-counter formulations (Fredholm *et al.,* 1999). Pharmacological actions of caffeine are related to its ability to block adenosine A1, $A2_A$ and $A2_B$ receptors (Fredholm *et al.,* 1999). Caffeine can also inhibit phosphodiesterase, promote Ca^{2+} release and block $GABA_A$ receptors, but very high concentrations are required to elicit these effects (Fredholm *et al.,* 1999). Caffeine exhibits adjuvant properties when added to analgesic drugs by increasing the analgesic effect of the drug (Sawynok, 2011) . Owoyele *et al*. (2004), demonstrated that caffeine also exhibited anti-nociceptive effect at 2.5mg/kg, 5mg/kg and 10mg/kg. While the only report on direct effect of caffeine on neuropathic pain was that of Wu *et al*. (2006) which stated that acute but not long term, caffeine intake reduced neuropathic pain state in nerve- injured rat.

Caffeine has been shown to act as adjuvant to acetaminophen when administered together and according to Sawynok, (2011) it has been co administered with several analgesic agents in order to test whether it would enhance the resulting analgesic effects. Despite the number of studies on the effects of taurine or caffeine alone on pain related behaviour in chronic, inflammatory and neuropathic pain models, as far as we know, there is no single report on the effect of co-administration of taurine and caffeine on neuropathic pain. Therefore we hypothesised that co-administration of taurine and caffeine could attenuate thermal hyperalgesia in chronic constriction injury model of neuropathic pain. This study was also aimed at investigating the possible involvement of autonomic receptor mechanism in the effects of taurine and its combination with caffeine.

MATERIALS AND METHODS

Drugs and reagents

Taurine and caffeine; MDA kits, hexamethonium and atropine were products of Sigma Chemical Co. (St. Louis MO, U.S.A) while propranolol and prazosin were products of Namco Chemicals, Amritsr, India. Normal saline and ketamine were purchased from Momrota pharmacy, opposite University of Ilorin Teaching Hospital's gate.

Experimental animals

A total of sixty six age matched male Wistar rats (*Rattus norvegicus*) weighing about 130±12.5g were employed in the present study. They were placed under standard laboratory condition, maintained on a normal light-dark cycle in cages at the animal holding of our faculty. They were fed on standard chow diet and water *ad libitum* and were acclimatized to laboratory conditions for two weeks before the commencement of experiments. The animals were treated in accordance with the ethical guidelines of the university and the guidelines agreed with the internationally accepted principles for animal handling and care.

Experimental protocol

Animals were randomly divided into two study groups (1 and 2 respectively). There were 8 sub-groups (A-H) in study group 1 and 4 sub- groups (I-L) in study group 2. Animals in the study group 1 were used to investigate the effect of combined systemic administration of taurine and caffeine on thermal hyperalgesia in sciatic nerve ligated rats while those in study group 2 were used to investigate the possible involvements of autonomic receptors in the combined effects of taurine and caffeine. The details of the groupings are as follows:

Group A (control): Rats were not subjected to any surgical procedure or treatment and were kept for 14 days. Animals in this group received normal saline (2ml/kg, i.p).The behavioural tests were employed on days 7 and 14. Thereafter on day 14, the animals were sacrificed and the biochemical estimations were performed.

Group B (Sham control): Rats were subjected to the surgical procedure to expose the left sciatic nerve without any nerve ligation, they also received normal saline (2ml/kg) for 14 days. The behavioural tests were employed on days 7 and 14. The animals were sacrificed on day 14 and the biochemical estimations were performed.

Group C (ligated control): Rats were subjected to the surgical procedure to expose and ligate the left sciatic nerve. The behavioural tests and the biochemical analysis were carried out. Animals in this group also received normal saline (2ml/kg, i.p).

Group D: Rats in this group received taurine (200mg/kg, i.p) alone for two weeks. Behavioural tests and biochemical analysis were also carried out as described for previous groups.

Group E: Rats in this group received caffeine (15mg/kg, i.p) alone for two weeks. Behavioural tests and biochemical analysis were also carried out as described for other groups.

Group F: Rats were subjected to sciatic nerve ligation and treated with taurine (100mg/kg, i.p) + caffeine (7.5mg/kg, i.p) for two weeks. The behavioural tests and the biochemical analysis were also done.
Group G: Rats were subjected to sciatic nerve ligation and treated with taurine (200mg/kg, i.p) + caffeine (15mg/kg, i.p) for two weeks. The behavioural tests and the biochemical analysis were also done.
Group H-L: Same as for group F but animals were pre-treated with propranolol (30mg/kg, i.p), Hexamethonium (10mg/kg, i.p), atropine (2mg/kg, i.p) and prazozin (5mg/kg, i.p) respectively.

Induction of neuropathic pain

Neuropathic pain was induced through chronic constriction injury in line with the modified method of Bennette and Xie, (1998). In brief, each rat was deeply anesthetized with 50mg/kg ketamine and the skin of the lateral surface of the left thigh was incised and a cut was made directly through the biceps femoris muscle to expose the sciatic nerve Once exposed, the sciatic nerve was tightly ligated with silk 4-0 thread at two sites with about 1mm gap (Kumar *et al.,* 2010). The silk suture was used instead of chromic gut suture as it has been documented that the chromic gut suture initiates inflammatory reactions in the sciatic nerve. Extra care was taken to avoid interruption of epineural blood flow while tying the ligature. In Sham operated animals, the same surgical procedure was also followed; the connective tissues were freed and ligatures were not applied. After performing the ligation, muscular and skin layer was immediately sutured with thread and topical antibiotic was applied. Nociceptive threshold was assessed at weekly intervals on days 7 and 14 after the surgery as suggested by Kumar *et al*. (2010).

Tail Heat Hyperalgesia Test

Thermal heat hyperalgesia was assessed by the tail immersion test as described by Necker and Hellon (1978). Tail heat-hyperalgesia was noted with the immersion of terminal part of the tail (1cm) in water, maintained at a temperature of 52.5±0.50°C. The tail withdrawal latency (TWL) was recorded, as a response to heat thermal sensation, and a cut-off time of 15s was maintained.

Tail Cold Hyperalgesia

Thermal cold sensitivity was assessed by the tail immersion method as described by Necker and Hellon (1978). Tail cold hyperalgesia was noted with the immersion of terminal part of the tail (1cm) in water maintained at a temperature of about 4°C. The tail withdrawal latency was recorded as a response of cold thermal sensation and a cut-off time of 15s was maintained.

Paw heat-hyperalgesia (hot plate test)

The thermal heat nociceptive threshold, as an index of thermal hyperalgesia, was assessed by using a hot plate, maintained at temperature of 52.5±1.0°C. The rat was placed on the hot plate and withdrawal latency, with respect to jumping off the hot plate, was recorded in seconds. The cut-off time of 15s was maintained.

Assessment of oxidative stress in sciatic nerve

Animals were euthanized with 50mg/kg ketamine on the 14th day, sciatic nerve was immediately isolated and homogenized in 50mM phosphate buffer (pH 7.4). The homogenate was centrifuged at 1000rpm for 10min and the supernatant was used for estimation of tissue MDA concentration.

Determination of MDA concentration

The assay method of Gutteridge and Wilkins (1982) was adopted. Malondialdehyde, a product of lipid peroxidation, when heated with 2-thiobarbituric acid (TBA) under acid conditions forms a pink colored product which has a maximum absorbance of 532 nm. Two mililitre of 0.7% TBA and 1 ml of glacial acetic acid were added to 2 ml of serum. The mixture was thoroughly mixed and incubated in water bath at 80°C for 20 min. It was then allowed to cool and centrifuged at 400 rev/min for 10 min. Absorbance of the supernatant was read at 532 nm against a blank wherein homogenates was substituted with distilled water. The results were expressed as nanomoles MDA/ml.

Statistical analysis

The results were expressed in mean ± standard error of the mean (SEM). The results were analyzed using one way Analysis of variance (ANOVA), followed by DUNCAN post hoc test. P value <0.05 was considered to be statistically significant.

RESULTS

Effect of combined administration of taurine and caffeine on thermal hyperalgesia and the effect of pre-treatment with autonomic receptor antagonists

Chronic constriction injury to the sciatic nerve resulted in significant development of heat hyperalgesia compared to the ligated control group, assessed by employing the tail immersion tests and hot plate test. Combined systemic administration of taurine (100mg/kg, i.p) and caffeine (7.5mg/kg, i.p)

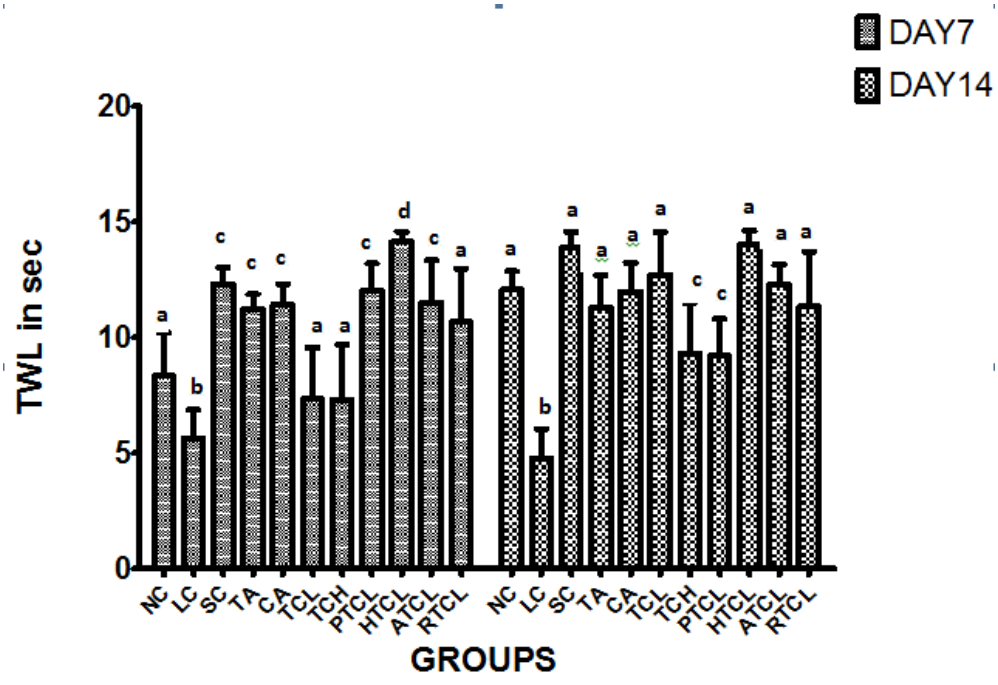

Fig. 1: Effect of combined administration of taurine and caffeine on tail cold hyperalgesia and the effect of pre-treatment with autonomic receptor blockers.in rats. Bars with the same letterings are not significantly different from each other (for each day). NC- non ligated control, LC- ligated control, SC- sham operated control, TA- taurine alone, CA- caffeine alone, TCL- low dose of taurine and caffeine, TCH- high dose of taurine and caffeine, PTCL- propranolol+ low dose of taurine and caffeine, HTCL- hexamethonium+ low dose of taurine and caffeine, ATCL- Atropine+ low dose of taurine and caffeine, Prazosin + low dose of taurine and caffeine.Data were expressed as mean ± SEM and n=6.

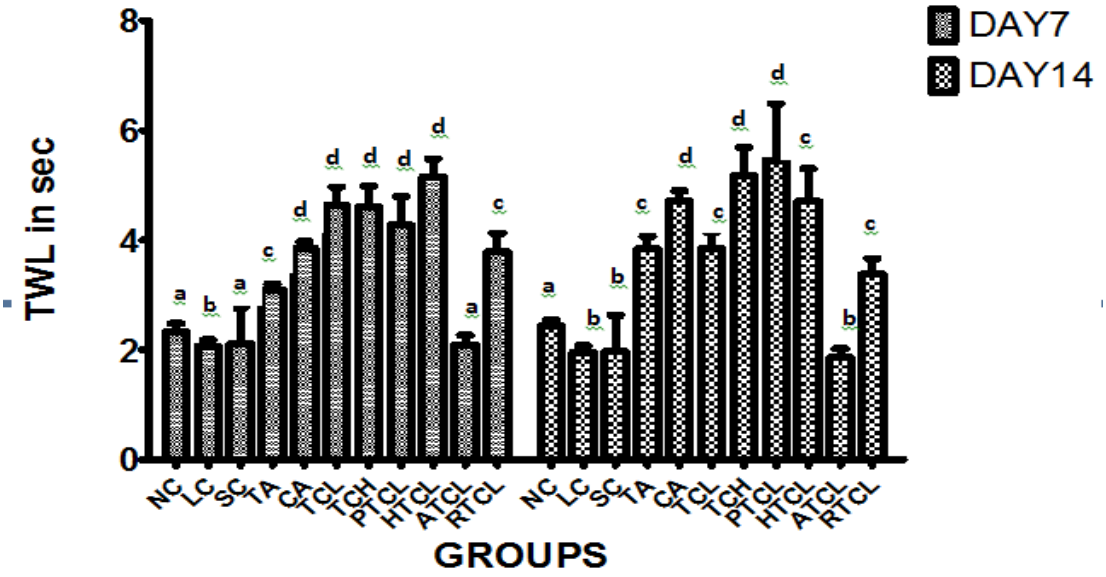

Fig. 2: Effect of combined administration of taurine and caffeine on tail heat hyperalgesia and the effect of pre-treatment with autonomic receptor blockers.in rats. Bars with the same letterings are not significantly different from each other (for each day). NC- non ligated control, LC- ligated control, SC- sham operated control, TA- taurine alone, CA- caffeine alone, TCL- low dose of taurine and caffeine, TCH- high dose of taurine and caffeine, PTCL- propranolol+ low dose of taurine and caffeine, HTCL- hexamethonium+ low dose of taurine and caffeine, ATCL- Atropine+ low dose of taurine and caffeine, Prazosin + low dose of taurine and caffeine. Data were expressed as mean ± SEM and n=6.

Table 1:Effect of combined administration of taurine and caffeine on MDA level in the sciatic nerve homogenate.

GROUP	MDA level (nM/ml)
Control	2.54±0.32[a]
Ligated control	4.42±0.61[b]
Sham operated Control	2.40±0.18[a]
Taurine (200mg/kg, i.p)	3.00±0.23[a]
Caffeine (15mg/kg, i.p)	4.53±0.52[b]
Taurine and caffeine (low dose)	3.13±0.65[a]
Taurine and caffeine (high dose)	2.95±0.12[a]

Columns with the same letterings are not significantly different from each other. n=6 for all groups.

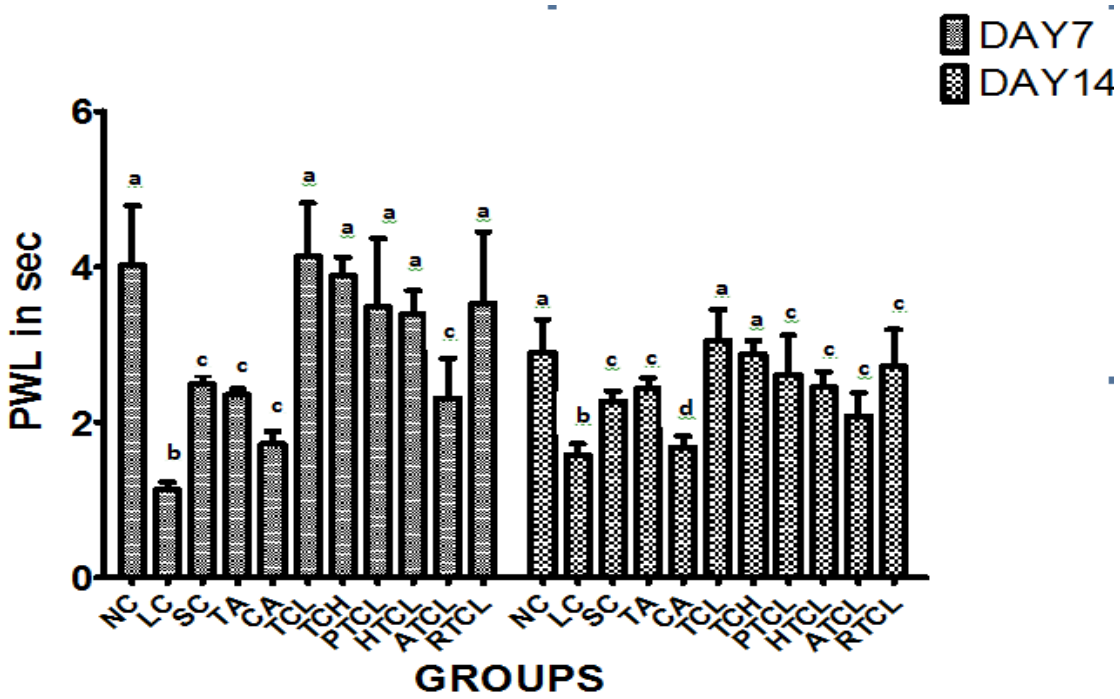

Figure 3: Effect of combined administration of taurine and caffeine on paw heat hyperalgesia and the effect of pre-treatment with autonomic receptor blockers.in rats. Bars with the same letterings are not significantly different from each other (for each day). NC- non ligated control, LC- ligated control, SC- sham operated control, TA- taurine alone, CA- caffeine alone, TCL- low dose of taurine and caffeine, TCH- high dose of taurine and caffeine, PTCL- propranolol+ low dose of taurine and caffeine, HTCL- hexamethonium+ low dose of taurine and caffeine ,ATCL- Atropine+ low dose of taurine and caffeine, Prazosin + low dose of taurine and caffeine.Data were expressed as mean ± SEM and n=6.

significantly attenuated the thermal hyperalgesia as shown by increase in tail and paw withdrawal latencies in treated groups compared to the ligated control group ($p<0.05$) (figures 1-3). Pre-treatment with atropine (2mg/kg, i.p) reversed the observed effect of combined administration of taurine and caffeine. Hexamethonium also abolished the antinociceptive effects of taurine and caffeine after two weeks in the hot plate test whereas the adrenergic antagonists did not abolish the effects of taurine and caffeine.

Effect of combined administration of taurine and caffeine on oxidative stress damage marker (MDA level)

CCI resulted into increase tissue MDA level in the sciatic nerve. However, treatment with taurine alone and in combination with caffeine (at the two doses) significantly reduced the MDA level compared to the ligated control group ($P<0.05$). Caffeine alone did not produce any significant effect on the tissue MDA level

compared with to the ligated control group (P>0.05) as shown in (table 1).

Discussion

In the present study, combined administration of taurine and caffeine attenuated thermal hyperalgesia induced by chronic constriction injury to the sciatic nerve. Sciatic nerve ligation has been reported to play a major role in the pathogenesis of neuropathic pain (Bennett and Xie, 1998). Neuropathic pain is usually triggered by lesions to the somatosensory nervous system that alter its structure and function that results in spontaneous pain and responses to noxious and innocuous stimuli are pathologically amplified (Costigan *et al.,* 2009). Also it has been reported that no satisfactory therapeutic intervention is still available to treat sciatic nerve ligation-induced neuropathic pain. Thus, the present study was designed to explore the possible therapeutic strategy to prevent sciatic nerve ligation-induced neuropathic pain.

Terada *et al* (2011) , reported that chronic constriction injury to the sciatic nerve resulted in mechanical allodynia, mechanical hyperalgesia, and thermal hyperalgesia. This observation is supported by the fact that in the present study, rats subjected to sciatic nerve ligation showed significant (P<0.05) decrease in pain threshold compare to the groups treated with caffeine or taurine and in combination as shown in figures1-3.

Caffeine, when administered alone inhibited thermal hyperalgesia in this study and this further confirmed the findings of Wu *et al*, (2006) which stated that acute but not long term, caffeine intake reduced neuropathic pain state in nerve- injured rats. This could be due to the ability of caffeine to induce central cholinergic analgesia (Ghelardini *et al.,* 1997).

Treatment with taurine alone also significantly increased pain threshold which is in line with what has been reported by Terada *et al* (2011) and it was attributed to the ability of taurine to reduce spinal and supraspinal integrated pain responses.

Caffeine is known to act as adjuvant analgesic and it enhanced the effect of taurine in this study which is in line with its action on headache pain (Zhang, 2001). Caffeine induced antinociception is attributed to block of adenosine $A2_A$ and $A2_B$ receptors and such action may also contribute to adjuvant analgesia.

Pellicer *et al.* (2007) reported that taurine in the cingulate cortex interfered with the glycine receptor and induced antinociception. Taurine is known to bind with GABA and glycine receptors and both GABAergic and Glycinergic neurons in the dorsal horn of the spinal cord have emerged as the pivotal inhibitory modulators of spinal pain in inflammatory and neuropathic pain (Zeilhofer, 2005). Though, the present study does not investigate the role of GABA receptors in the attenuating effect of combined administration of taurine and caffeine on thermal hyperalgesia.

Various autonomic receptor antagonists were employed in this study to explore the possible involvement of autonomic nervous system. The autonomic receptor antagonists employed include: atropine, hexamethonium, propranolol and prazosin. Atropine, a muscarinic receptor antagonist abolished the antihyperalgesic effect of combined administration of taurine and caffeine in the tail heat hyperalgesia and hot plate tests as shown in Tables 2 and 3 suggesting the involvement of muscarinic system/cholinergic system. Analgesia via activation of muscarinic receptors has been described and it is known to be centrally mediated (Pedigo *et al.,* 1975). Though, the therapeutic use of direct muscarinic agonists clinically was never pursued, due to severe side effects. The nicotinc receptors could also be involved in the taurine-caffeine mediated antinociption as shown by its inhibitory effects after two weeks of ligation. The adrenergic mechanism seems not be involved in the anti nociceptive effects of the two substances but they may serve as adjuvants to them.

Moreover, sciatic nerve ligation has been reported to cause imbalance in reactive oxygen species (ROS) and antioxidant enzymes (Senoglu *et al.,* 2009). This is strongly supported by the fact that in the present study, sciatic nerve ligated rats showed high degree of oxidative stress which is expressed by high levels of lipid peroxidation i.e. increase in MDA level. Previous report by Xu *et al.*(2007) showed that oxidative stress occurs due to activation of p38MAPK pathway . In turn p38MAPK have been shown to increase the production of various inflammatory mediators such as TNF-α, ILs, COX-2 which are important mediators for the progression of neuropathic pain.(Raghavendra *et al.,* 2003) . However, we did not investigate the effects of caffeine and taurine on inflammatory mediators.

Combined administration of taurine and caffeine in the present study attenuated the ligation-induced increase in the level of lipid peroxidation within the sciatic nerve as shown in table 1. Taurine is found particularly in high concentrations in tissues exposed to elevated levels of oxidants, suggesting its role in the attenuation of oxidative stress (Green *et al.,* 1991). Thus, it is likely that taurine and caffeine when administered together decreased reactive oxygen species via the inhibition of p38 MAPK.

In conclusion, the present study showed that combined administration of taurine and caffeine inhibited thermal

hyperalgesia associated with sciatic nerve ligation which involves activation of muscarinic but not nicotinic receptors of the cholinergic system. There is also attenuation of oxidative stress and consequently an improvement of the normal function of sciatic nerve. Therefore, this study has shown that combination of taurine and caffeine can be useful for the treatment of neuropathic pain.

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