SECTION 6

DISCUSSION
The CCI model is the most commonly employed animal model of nerve damage-induced neuropathic pain (Bennett and Xie, 1988; Jaggi et al., 2011). In this model, the pain is induced by entrapping the sciatic nerve through four loose ligatures and it shares the pathophysiology of carpal tunnel syndrome and tarsal tunnel syndrome in humans due to entrapment of median nerve in narrowing carpal tunnel. Furthermore, this model has also been suggested to share the pathophysiology of complex regional pain syndrome in humans (Bennett and Xie, 1988; Jin et al., 2008). In the present study, the chronic constriction injury led to significant development of cold allodynia, mechanical hyperalgesia, heat hyperalgesia and mechanical allodynia assessed on 14th day after surgery. Furthermore, the spontaneous pain, assessed in terms of cumulative paw lifting and paw licking duration, and foot deformity was also pronounced on 14th day in CCI subjected rats. It has been reported that chronic constriction injury of the sciatic nerve causes dramatic alterations in the morphology and the physiology of the injured sciatic nerve and the neurons of DRG/dorsal horn of the spinal cord, with the maximal effects at approximately 2 weeks after nerve injury (Bennett and Xie, 1988; Kim et al., 1997; Jiang et al., 2008). In our previous studies, the peak behavioral alterations were reported on 14th day after the nerve injury in CCI model (Muthuraman et al., 2008). Therefore in the present study, the behavioral alterations were assessed on 14th day after chronic constriction injury.

In the present investigation, administration of spironolactone (10 and 20 mg/kg, p.o.), telmisartan (2 and 5 mg/kg, p.o.) and pregabalin (10 mg/kg, p.o.) for 14 days significantly attenuated CCI-induced behavioral alterations including paw cold allodynia; mechanical and heat hyperalgesia; mechanical allodynia; spontaneous pain and postural defect in terms of foot deformity. The various preclinical and clinical studies have documented the effectiveness of pregabalin in attenuating neuropathic pain. Clinically, it has been considered as one of the first line agents in neuropathic pain management (Bauer et al., 2009, Kumar et al., 2010). From our own laboratory, spironolactone has been reported to attenuate diabetic hyperalgesia in mice (Khan et al., 2009). Recently, spironolactone has been reported to attenuate acetic acid-induced inflammatory visceral pain in mice (Abdel-Salam et al., 2010). The clinical studies have also shown the effectiveness of angiotensin AT1 receptor blockers including telmisartan in preventing the
attacks of migraine (Diener et al., 2009; Gales et al., 2010). Furthermore, it has also been reported that the repeated administration of angiotensin AT$_1$ receptor blockers and angiotensin converting enzyme inhibitors such as spirapril, trandolapril and losartan exhibit anti-nociceptive actions in mice (Takai et al., 1996). However, it is first report suggesting the ameliorative potential of spironolactone and telmisartan in CCI-induced neuropathic pain in rats.

In the present study, CCI-induced neuropathic pain was also associated with rise in the TNF-α levels in the sciatic nerve. However, administration of spironolactone (10 and 20 mg/kg) and telmisartan (2 and 5 mg/kg) attenuated CCI-induced rise in the sciatic nerve TNF-α levels in a significant manner. Because TNF-α appears early in the cytokine cascade, therefore, it has been considered as a prototype pro-inflammatory mediator (Kang et al., 2006). The role of TNF-α has been well documented in peripheral as well central sensitization in neuropathic pain (Figure 25) (Leung and Cahill, 2010). An up-regulation of TNF-α has been detected at the injury site mainly in macrophages and Schwann cells (Wagner and Myers, 1996b). The nerve biopsies from patients with painful neuropathy also show higher levels of TNF-α expression, especially in the Schwann cells (Empl et al., 2001). Recently, the bilateral elevation of TNF-α has also been documented in the lumbar and cervical DRG after unilateral chronic constriction injury of the sciatic nerve (Jancálek et al., 2010). An intra-sciatic injection of TNF-α has been shown to produce pain hypersensitivity similar to that of neuropathic pain in humans (Sorkin and Doom, 2000) and neutralizing antibodies to TNF-α receptors attenuate pain hypersensitivity.

In recent years, spironolactone has been shown to improve the disease states in diabetic nephropathy; renal failure; endothelial dysfunction and inflammatory disease activity in rheumatoid arthritis; retinal vasculopathy and vascular stiffness (Syngle et al., 2009; Kithas and Supiano, 2010; Lin et al., 2010). In most of the studies, its beneficial effects have been attributed to its potent anti-inflammatory activity and ability to inhibit the production of different pro-inflammatory cytokines (Ikeda et al., 2009; Syngle et al., 2009). The studies have also documented the usefulness of telmisartan in different pathophysiological states including cardiac hypertrophy, endothelial dysfunction, chronic kidney disease, cognitive impairment, cerebral injury, myocardial injury and renal injury.
Its beneficial effects in these disease states has been ascribed secondary to inhibition of pro-inflammatory mediators (Fouad et al., 2010; Kasahara et al., 2010; Sukumaran et al., 2010). Based on these, it is possible to suggest that spironolactone and telmisartan mediated beneficial effects in neuropathic pain in the present study may be due to their TNF-α attenuating effect (Figure 26).

Angiotensin AT₁ receptors are present on the inflammatory cells (Kitazono et al., 1995; Potter et al., 1998) and the critical role of renin-angiotensin system in different processes of inflammation including accumulation of neutrophils; differentiation of dendritic cells and production of inflammatory chemokines has been described (Nahmod et al., 2003; Nabah et al., 2004). Telmisartan is reported to possess the highest affinity for the angiotensin AT₁ receptors (Kakuta et al., 2005) and stronger anti-inflammatory effects than other angiotensin AT₁ receptor antagonists (Kurtz and Pravenec 2004). An increased aldosterone levels have also been well documented to produce deleterious effects in different parts of the body through inflammatory reactions (Sun et al., 2002). The blockade of aldosterone action with spironolactone has been shown to inhibit the activation of NF-kB and inflammation (Kang et al., 2006). However, there have been reports suggesting that the suppressive effect of spironolactone on immune-reactive and inflammatory cytokines is independent of mineralo-corticoid receptors blockade (Sønder et al., 2006 a,b).

The CCI model in rodents has been described to mimic the complex regional pain syndrome in humans (Bennet and Xie, 1988; Jin et al., 2008), which is characterized by activation of sympathetic nervous system. The activation of sympathetic nervous system in turn stimulates renin-angiotensin-aldosterone system followed by activation of angiotensin AT₁ receptors and increased aldosterone secretion. Taken together, it may be proposed that nerve injury-induced activation of sympathetic nervous system leads to activation of renin-angiotensin system which in turn is associated with activation of angiotensin AT₁ receptors and release of aldosterone leading to initiation and maintenance of neuropathic pain (Figure 27).

Vincristine is an anti-neoplastic agent and is widely used in cancer therapy for both solid tumors and hematologic malignancies. However, it produces neurotoxicity to all patients predictably and limits its use in cancer treatment. Clinically, the neuropathy is
sensorimotor and starts with paresthesias in hands and feet (Jaggi and Singh 2012). The pinprick and temperature senses are more affected than vibration sense. Vincristine-induced neuropathy is mainly ascribed to its high binding affinity for tubulin, a protein subunit of microtubules, leading to dissolution of axonal microtubules. The studies have also suggested the changes in the axoplasmic flow due to vincristine treatment (Authier et al., 2003 a,b).

In the present investigation, administration of vincristine (50 µg/kg, i.p.) for 10 days led to development of cold allodynia, mechanical hyperalgesia, heat hyperalgesia and mechanical allodynia. The observed behavioral alterations in this study are in consistent with the earlier reports documenting the development of pain symptoms with vincristine administration (Siau and Bennett, 2006; Muthuraman et al., 2008; Kaur et al., 2010). However, the spontaneous pain and foot deformity was not observed in vincristine injected rats. Furthermore, vincristine administration did not produce elevation in TNF-α levels in the sciatic nerve indicating the lack of role of inflammatory reactions in the pathobiology of neuropathic pain. However in contrast, Kiguchi and co-workers have reported the role of pro-inflammatory mediators in the vincristine-induced neuropathic pain (Kiguchi et al., 2008) These contradictory results may probably be due to administration of higher dose of vincristine (100 µg/kg) by Kiguchi and co-workers as compared to lower dose of vincristine (50 µg/kg) employed in the present study. Administration of spironolactone (5, 10 and 20 mg/kg) and telmisartan (1, 2 and 5 mg/kg) for 14 days did not modulate vincristine associated pain related behavior suggesting the lack of their potential in modulating vincristine-induced neuropathic pain. However, administration of pregabalin (10 mg/kg) for 14 days significantly attenuated vincristine-induced pain manifestations. From the results of the present study, it seems that inflammation is not a critical factor in the pathogenesis of chemotherapy-induced neuropathic pain and therefore, the involvement of non-inflammatory mechanisms in its pathogenesis may probably explain the lack of beneficial effect of spironolactone and telmisartan in chemotherapy associated pain.

In the present investigation, single intra-thecal injection of FTS (5 and 10 µg) and pregabalin (100 µg) attenuated CCI and vincristine-induced pain manifestations on 14th day at different time periods such as 30 min, 60 min, 120 min and 180 min after its
administration with the peak anti-nociceptive effects at 60 min. However, its analgesic effects tend to wean off after 60 min and the diminished anti-nociceptive effects were observed at 180 min after its administration. The studies have shown the neuroprotective effects of FTS in traumatic head injury (Shohami et al., 2003; Marciano et al., 2007) and experimental autoimmune encephalomyelitis (Aizman et al., 2010) along with attenuation of the bovine myelin-induced experimental autoimmune neuritis, a model of inflammatory neuropathy (Kafri et al., 2005). However, it is the first report demonstrating the analgesic potential of FTS in CCI and vincristine-induced neuropathic pain in rats.

FTS is a novel Ras inhibitor and its potent analgesic effects noted in the present study may possibly be attributed to inhibition of Ras mediated signaling pathway. Ras constitutes an important part of intracellular signal transduction pathway involving MAPK family as Ras/Raf/MEK/ERK2 cascade and it triggers the activation of MAP kinase signaling pathway. A large number of studies have suggested the critical role of MAP kinase family including ERK, p38 kinase and JNK in different models of neuropathic pain and intra-thecal administration of different p38 inhibitors and MEK inhibitors have been shown to attenuate the painful manifestations of neuropathic pain in different animal models (Tsuda et al., 2004; Zhuang et al., 2005). Furthermore, the studies have shown the critical role of members of Ras super family such as Rho-kinase (ROCK) (Ramer et al., 2004) and homolog of Ras such as Rheb in different forms of pain including neuropathic pain (Norsted Gregory et al., 2010). The studies have also suggested the cross-talk between Ras and Rho signaling pathways (Sahai et al., 2001). Recently, administration of fasudil, a ROCK inhibitor, has been shown to produce analgesic actions in different preclinical models of pain including spinal-nerve ligation and CCI-induced neuropathic pain; capsaicin-induced secondary mechanical hypersensitivity and sodium iodoacetate-induced osteoarthritis pain (Boyce-Rustay et al., 2010). Furthermore, Rho inhibitor (C3 toxin) and ROCK inhibitors (fasudil and Y-27632) have been shown to prevent in vitro as well as in vivo neuronal degeneration following methyl mercury exposure suggesting the role of Rho/ROCK pathway in the axonal degeneration and apoptotic neuronal cell death (Fujimura et al., 2011). Other
studies have also documented the analgesic potential of ROCK inhibitors in inflammatory, diabetic and neuropathic pain (Ohsawa et al., 2011).

In the present investigation, single intra-thecal administration of GW 5074 (a selective c-Raf1 kinase inhibitor) also attenuated CCI and vincristine-induced different pain manifestations on 14th day at different time intervals such as 30 min, 60 min, 120 min and 180 min with the peak anti-nociceptive effects at 60 min after its administration. However, its analgesic effects were significantly diminished at 180 min suggesting the reversibility of anti-nociceptive actions. Earlier studies have shown the potential usefulness of GW 5074 in producing in vitro as well as in vivo neuroprotection in response to different neurotoxic stimuli and in attenuating neurodegenerative disorders like Alzheimer’s and Huntington’ diseases (Echeverria et al., 2008). However, it is the first report documenting the potential of GW 5074 in attenuating pain manifestations in experimental models of neuropathic pain. The studies have shown that the pathogenesis of neuropathic pain and morphine tolerance share similarities at the molecular levels (Mao et al., 1995; Mayer et al., 1999) and reports have documented c-Raf1 as important target in attenuating morphine hyperalgesia as well as tolerance. GW5074 is reported to attenuate morphine-mediated augmented CGRP release during morphine hyperalgesia suggesting that morphine-mediated increase in pain sensitization is linked to augmentation of CGRP release from primary nerve afferents in a Raf-1 dependent manner (Yue et al., 2008; Tumati et al., 2009). Furthermore, it has been shown that the knockdown of spinal Raf-1 levels in vivo by intrathecal administration of Raf-1-specific selective small interfering RNA (siRNA) attenuate sustained morphine-mediated thermal hyperalgesia and anti-nociceptive tolerance in rats (Tumati et al., 2008; 2010). Based on these, it is suggested that FTS and GW 5074 have the potential to attenuate hyperalgesia and allodynia in CCI and vincristine-induced neuropathic pain in rats that may probably be linked to inhibition of Ras and c-Raf 1 as a part of intracellular signal transduction pathway involving MAPK family (Figure 28).
Figure 25: Possible mechanisms responsible for TNF-α-induced neuronal sensitization leading to induction and maintenance of neuropathic pain. TNFR1 and TNFR2: TNF-α receptors; MAPK: Mitogen activated protein kinase; NF-kB: Nuclear factor kappa B; IL-6: Interleukin-6; NGF: Nerve growth factor.
Figure 26: Effects of spironolactone and telmisartan on CCI and vincristine-induced peripheral neuropathic pain.
Figure 27: Proposed mechanisms involved in development of neuropathic pain in CCI model.
Figure 28: Analgesic effects of FTS and GW5074 in CCI and vincristine models of neuropathic pain.