CHpATER 4

Protective mechanism of resveratrol, montelukast and their interaction in spinal nerve ligation induced neuropathic pain: Possible involvement of noradrenaline and serotonin

4.1. Introduction

Neuropathic pain is one of the chronic debilitating form of pain and comorbid with disease conditions like depression, diabetes, cancer chemotherapy, HIV and other viral infections (Haanpaa et al. 2010). Complex pathology of neuropathic pain and its poor understanding make it further difficult to develop suitable therapeutic strategies for the effective pain relief. Therefore, there is imperative need to develop drugs that provide sufficient pain relief. Both depression and pain shares common pathology to some extent which made to think about the role of antidepressants in chronic pain therapy (Micó et al. 2006; Miller and Cano 2009). Further, different antidepressants have also been claimed to be first line agents owing to their effects on descending monoamine pathway and receptor binding profiles (Attal et al. 2010; Dworkin et al. 2010).

Persistent peripheral nerve injury stimulation leads to central sensitization. Descending pathway, starting from supraspinal regions (mid brain and brain stem) ending in spinal cord has been shown to play a pivotal role in overall sensory processing. Both descending facilitatory and an inhibitory pathways are differentially regulated in various painful conditions including NP (Ossipov et al. 2010). Under normal physiological conditions, descending inhibitory pathways play a protective role by recruiting endogenous analgesic mediators like GABA thereby reduce painful stimulation (Gassner et al. 2009). But it becomes maladaptive in neuropathy and maintains the painful state owing to their imbalance between inhibitory and facilitatory pathways (Kwon et al. 2014). Major neurotransmitters involved in this descending pathway are noradrenaline (NA) and serotonin (5HT). Besides, various preclinical studies clearly state that both NA (through α2-adrenoceptors) and 5HT (through 5HT7 receptors) acts at spinal levels to mediate inhibitory actions (Dogrul et al. 2009; Kwon et al. 2014). Different category of drugs like pregabalin, tricyclic antidepressants (TCAs), tramadol and newer drug like tapentadol have been well shown to alter one or
both these neurotransmitters and neuroplasticity at spinal & supraspinal levels (Bymaster et al. 2001; Niesters et al. 2014; Rahman et al. 2009; Yamasaki et al. 2015).

Endogenous opioid system (via mu, delta and kappa receptors) plays a key role downstream to descending inhibitory control in antidepressant mediated antinociception (Benbouzid et al. 2008). Besides, these endogenous opioid pathways and lipoxigenase (LOX) products works in close approximation in spinal cord (Christie et al. 2000; Trang et al. 2003). Owing to the relation between this descending monoamine pathway, endogenous opioid system and LOX pathways, we have hypothesised to elucidate the interaction between descending monoamine and LOX pathways if any in experimental models of chronic pain. Besides, patients who are on montelukast therapy are advised to take more doses of antidepressants that raises a hope for possible interaction between them (Zhou et al. 2013). Leukotrienes (LTs) are derived from arachidonic pathways via LOS action. Recently, change in LT receptors expression and its involvement in neuropathic pain have been observed in neuropathic pain models (Okubo et al. 2010).

Resveratrol has been well known to exert its potent antioxidant, antiinflammatory effects in different neurodegenerative conditions (Kumar et al. 2007). Besides, Resveratrol has been reported to enhance the monoamine transmission in depressive subjects and exerted antidepressant like actions (Huang et al. 2013; Xu et al. 2010). Even though resveratrol is effective in depressive subjects however, its exact mechanism in neuropathic pain is not fully understood and needs further investigation.

Besides, montelukast a cysteinyl leukotriene receptor antagonist has been shown to exert its neuroprotective effects in different neurodegenerative conditions like Huntington’s disease (Kalonia et al. 2010), traumatic brain injury (Biber et al. 2009) in cerebral ischemia (Muthuraman et al. 2012; Saad et al. 2015; Zhou et al. 2014). Many studies claimed the protective effects of montelukast because of its antioxidant and neuroinflammatory like effects (Coskun et al. 2011; Dengiz et al. 2007; Kalonia et al. 2010; Saad et al. 2015; Zhou et al. 2014). Besides, 5-LOX deficiency has been shown be associated with antidepressant activity (Uz et al. 2008). Further, synergistic interaction between opioids and COX/specific 5-LOX
inhibitors have also been observed at supraspinal regions (periaqueductal grey) indicating the possible role of LOX pathway in centrally mediated analgesic actions (Christie et al. 1999; Vaughan et al. 1997; Zhang and Pan 2012).

Depletion of monoamine levels forms the common pathway between depression and pain. Further, many of the antidepressants acting via monoamine pathways have been shown effective clinically in chronic pain but limit their use because of different adverse effects (Bymaster et al. 2001; Sindrup et al. 2005). Besides, clinical study also suggests that montelukast therapy is associated with an increased need of antidepressants (Zhou et al. 2013). Further, 5-LOX and their metabolites are associated with depression and its deficiency has been correlated with antidepressant activity in animal experiments (Dzitoyeva et al. 2008; Manev and Manev 2007; Uz et al. 2008). Based on potential effect of resveratrol on monoamine pathway and association of depression with montelukast, it would be essential to check the relationship of these drugs on monoamine pathways in chronic painful conditions.

Hence, the current study has been undertaken to explore the protective effect of resveratrol and its interaction with montelukast, targeting descending monoamine pathway against SNL induced neurobehavioral, biochemical, neurotransmitter and cellular alterations in rats.

4.2. Materials and Methods

4.2.1. Animals

Male SD rats (180-220 g), obtained from Central Animal House, Panjab University, Chandigarh were utilised in the current protocol. The animals were maintained on 12-h light/dark cycle, with food and water ad libitum. Ethical clearance from IAEC of Panjab University was obtained prior to the start of the experiment (IAEC/536/UIPS, 10/20/01/15) and conducted as per the Indian National Science Academy guidelines for the use and care of experimental animals.

4.2.2. Spinal nerve ligation

Refer to chapter 2 (2.2.2)
4.2.3. Drug and treatment schedule

The present study (Fig. 4.1) includes eleven treatment groups (n = 8) (Table 4.1). Resveratrol (Sigma Chemicals, St. Louis, USA) suspended in 0.25% w/v CMC and montelukast (Dr. Reddy’s Laboratories, India) dissolved in normal saline. Both the drugs were administered intraperitoneally (i.p.) as per body weight (5 ml/kg) to all the treatment groups daily in the morning 10:00 h, for 28 days starting from the day after SNL surgery. Doses were selected on the basis of previously published reports (Kalonia et al. 2010; Kumar et al. 2007).

Figure 4.1 Experimental protocol

4.2.4. Behavioural Assessments

4.2.4.1. Mechanical allodynia
Refer to chapter 1 (1.2.4.3)

4.2.4.2. Mechanical hyperalgesia
Refer to chapter 1 (1.2.4.4)

4.2.4.3. Cold allodynia
Refer to chapter 2 (2.2.4.3)
Table 4.1 Treatment groups

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Group</th>
<th>Treatment (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Naive</td>
<td>Healthy animals (no treatment)</td>
</tr>
<tr>
<td>2</td>
<td>Sham</td>
<td>Surgery performed, vehicle administered</td>
</tr>
<tr>
<td>3</td>
<td>Control (SNL)</td>
<td>SNL + vehicle administered for 28 days</td>
</tr>
<tr>
<td>4</td>
<td>SNL + Res (5)</td>
<td>SNL + Resveratrol (5 mg/kg, i.p.)</td>
</tr>
<tr>
<td>5</td>
<td>SNL + Res (10)</td>
<td>SNL + Resveratrol (10 mg/kg, i.p.)</td>
</tr>
<tr>
<td>6</td>
<td>SNL + Res (20)</td>
<td>SNL + Resveratrol (20 mg/kg, i.p.)</td>
</tr>
<tr>
<td>7</td>
<td>SNL + Mon (0.5)</td>
<td>SNL + Montelukast (0.5 mg/kg, i.p.)</td>
</tr>
<tr>
<td>8</td>
<td>SNL + Mon (1)</td>
<td>SNL + Montelukast (1 mg/kg, i.p.)</td>
</tr>
<tr>
<td>9</td>
<td>SNL + Mon (2)</td>
<td>SNL + Montelukast (2 mg/kg, i.p.)</td>
</tr>
<tr>
<td>10</td>
<td>SNL + Res (5) + Mon (0.5)</td>
<td>SNL + Resveratrol (5 mg/kg, i.p.) + Montelukast (0.5 mg/kg, i.p.)</td>
</tr>
<tr>
<td>11</td>
<td>SNL + Res (10) + Mon (1)</td>
<td>SNL + Resveratrol (10 mg/kg, i.p.) + Montelukast (1 mg/kg, i.p.)</td>
</tr>
</tbody>
</table>

4.2.5. Biochemical assessments

4.2.5.1. Tissue collection and homogenization

Following behavioural assessments, animals were sacrificed by cervical dislocation and lumber spinal cord (L5-L6) regions were dissected out. The spinal cord sections of the one group (n=5) were used for the assessment of oxidative stress parameters and the other group (n=3) was used for neurotransmitter estimations. For antioxidant enzyme activity, a 10% (w/v) tissue homogenates in 0.1 M phosphate buffer (pH 7.4) were prepared. The homogenates thus obtained were centrifuged at 10,000 × g at 4 °C for 15 min. Aliquots of supernatants obtained were assayed for various biochemical tests.
4.2.5.2. Measurement of endogenous antioxidant profile

4.2.5.2.1. Measurement of lipid peroxidation
Refer to chapter 1 (1.2.7.1.1)

4.2.5.2.2. Estimation of nitrite
Refer to chapter 2 (2.2.5.2.2)

4.2.5.2.3. Superoxide dismutase activity
Refer to chapter 1 (1.2.7.1.3)

4.2.5.2.4. GSH assay
Refer to chapter 1 (1.2.7.1.2)

4.2.5.2.5. Protein estimation
Refer to chapter 1 (1.2.7.1.5)

4.2.6. Spinal cord monoamine assessment

Neurotransmitter [noradrenaline (NA) and serotonin (5-HT)] levels were assessed in spinal cord regions by HPLC-ECD (Waters Delta 600 system controller equipped with an electrochemical detector (Waters 2465). The HPLC system consisted of a high pressure isocratic pump (Waters 600 solvent delivery pump), a 20 µl sample loop, symmetry®RP-C18 column (Waters, 4.6 × 250 mm; 5 µm). Mobile phase is a mixture of citrate buffer (0.1 M citric acid, 25 mM NaH₂PO₄, 25 mM EDTA, and 2 mM of 1-octane sulphonic acid)-methanol (87:13 v/v, pH 4.5). Detector conditions were maintained at +0.75 V with sensitivity ranging from 5 to 50 nA throughout the analysis. Samples (20 µl) were injected manually and flow rate has been maintained at 0.8 ml/min (Patel et al. 2005). The spinal cord samples were homogenized in a tissue homogenizer fitted with a teflon pestle rotating at high speed (Remi, Mumbai, India) in 0.2 M perchloric acid followed by centrifugation at 12000 × g for 5 min at 4 °C. 0.2mm nylon filters were used to filter the supernatant and the filtrate was injected to the HPLC by hamilton syringe. Waters Empower (2002) software was used to record & analyze the data.

4.2.7. Statistical analysis
Refer to chapter 1 (1.2.8)
4.3. Results

4.3.1. Effect of resveratrol, montelukast and their combination on mechanical allodynia of SNL treated rats

No significant (p<0.05) difference in mechanical allodynia was observed between sham and naive group animals. SNL significantly caused mechanical allodynia as evidenced by decrease in paw withdrawal threshold against vonfrey stimuli as compared to sham group. Resveratrol (10, 20 mg/kg), montelukast (1, 2 mg/kg) treatment for 28 days significantly augmented paw withdrawal threshold (Fig. 4.2) as compared to SNL control.

![Figure 4.2 Effect of resveratrol, montelukast and their combination on mechanical allodynia in SNL induced neuropathic pain.](image)

Data were expressed as mean ± S.E.M. a\(p<0.05\) compared to sham group; b\(p<0.05\) compared to SNL group; c\(p<0.05\) compared to Res (5); d\(p<0.05\) compared to Res (10); e\(p<0.05\) compared to Mon (0.5); f\(p<0.05\) compared to Mon (1); (Two way ANOVA followed by Bonferroni posttests). SNL: spinal nerve ligation; Res: resveratrol; Mon: montelukast.

However, lower doses of resveratrol (5 mg/kg) and montelukast (0.5 mg/kg) did not show any significant effect on mechanical allodynia as compared to SNL control group. Moreover, administration of resveratrol (5 & 10 mg/kg) in combination with montelukast (0.5 & 1 mg/kg) significantly improved paw
withdrawal threshold as compared to their effects per se in SNL treated animals. Further, per se effects of resveratrol (20 mg/kg) and montelukast (2 mg/kg) treatment did not demonstrate any significant effect on mechanical allodynia as compared to sham group animals (data not shown).

4.3.2. Estimation of resveratrol, montelukast and their combination on mechanical hyperalgesia

There is no significant ($p<0.05$) difference in mechanical hyperalgesia was observed between sham and naive group animals. SNL significantly caused mechanical hyperalgesia as evidenced by decrease in paw withdrawal threshold as compared to sham treated group.

![Mechanical Hyperalgesia](image)

**Figure 4.3** Effect of resveratrol, montelukast and their combination on mechanical hyperalgesia in SNL induced neuropathic pain. Data were expressed as mean ± S.E.M. $^a p < 0.05$ compared to sham group; $^b p < 0.05$ compared to SNL group; $^c p < 0.05$ compared to Res (5); $^d p < 0.05$ compared to Res (10); $^e p < 0.05$ compared to Mon (0.5); $^f p < 0.05$ compared to Mon (1); (Two way ANOVA followed by Bonferroni posttests). SNL: spinal nerve ligation; Res: resveratrol; Mon: montelukast.
Resveratrol (10, 20 mg/kg), montelukast (1, 2 mg/kg) treatment significantly improved paw withdrawal threshold (Fig. 4.3) as compared to SNL control. However, lower doses of both resveratrol (5 mg/kg) and montelukast (0.5, mg/kg) did not show any significant effect on mechanical hyperalgesia as compared to SNL control. Moreover, resveratrol (5 and 10 mg/kg) in combination with montelukast (0.5 and 1 mg/kg) showed significant improvement in paw withdrawal threshold on day 28 as compared to their individual effects in SNL treated rats. Further, per se effects of resveratrol (20 mg/kg) and montelukast (2 mg/kg) treatment did not demonstrate any significant effect on mechanical hyperalgesia as compared to sham group animals (data not shown).

4.3.3. Estimation of resveratrol, montelukast and their combination on cold allodynia

No significant ($p<0.05$) difference in cold allodynic response was observed between sham and naive group animals. SNL significantly resulted in cold allodynia as evidenced by increase in paw lifting and licking duration in response to acetone treatment as compared to sham group.

**Figure 4.4** Effect of resveratrol, montelukast and their combination on cold allodynia in SNL induced neuropathic pain. Data were expressed as mean ± S.E.M. $^a p < 0.05$ compared to sham group; $^b p < 0.05$ compared to SNL group; $^c p < 0.05$ compared to Res (5); (Two way ANOVA followed by Bonferroni posttests).

SNL: spinal nerve ligation; Res: resveratrol; Mon: montelukast.
Chapter 4

Chronic resveratrol (10 & 20 mg/kg), montelukast (2 mg/kg) treatment significantly attenuated paw licking and lifting duration (Fig. 4.4) as compared to SNL control. However, lower doses of both resveratrol (5 mg/kg) and montelukast (0.5 & 1 mg/kg) did not demonstrate any significant effect on cold allodynia as compared to SNL control. Moreover, combination of resveratrol (5 & 10 mg/kg) with montelukast (0.5 & 1 mg/kg) did not demonstrate any significant reversal effect on cold allodynia as compared to their effect per se in SNL treated rats. Further, resveratrol (20 mg/kg) and montelukast (2 mg/kg) per se treatment did not demonstrate any significant effect on cold alldynia as compared to sham group animals (data not shown).

4.3.4. Effect of resveratrol, montelukast and their combination on spinal cord oxidative damage (lipid peroxidation, nitrite, GSH, and SOD levels) in SNL treated animals

No significant difference in spinal cord oxidative stress parameters was observed between sham and naive group animals. SNL treatment significantly increased LPO, nitrite, depleted GSH and SOD enzyme activities in spinal cord as compared to sham group. Chronic treatment with resveratrol (10 & 20 mg/kg), montelukast (1 & 2 mg/kg) for 28 days significantly attenuated LPO, nitrite, restored GSH and SOD enzyme (p < 0.001) activities as compared to SNL control (Table 4.2). Whereas, resveratrol (5 mg/kg), montelukast (0.5 mg/kg) did not show any significant effect on the above mentioned oxidative stress parameters as compared to SNL control. However, combination of resveratrol (5 & 10 mg/kg) with montelukast (0.5 & 1 mg/kg) treatment significantly caused antioxidant like effect (attenuated LPO, nitrite, restored GSH and SOD enzyme activities) as compared to their effects per se in SNL treated animals. Further, resveratrol (20 mg/kg) and montelukast (2 mg/kg) per se treatment did not show any significant effect on oxidative stress parameters as compared to sham group animals (data not shown).
Table 4.2 Effect of resveratrol, montelukast and their combination spinal cord oxidative damage (lipid peroxidation, nitrite, GSH and superoxide dismutase activity) in SNL treated rats

<table>
<thead>
<tr>
<th>Treatment (mg/kg)</th>
<th>MDA nmol MDA/mg of protein (% of sham)</th>
<th>Nitrite µmol/mg of protein (% of sham)</th>
<th>GSH (nM/mg protein) (%Sham)</th>
<th>SOD Units/mg of protein (% of sham)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naive</td>
<td>2.52 ± 0.1 (111.9)</td>
<td>29.11 ± 2.0 (95.44)</td>
<td>56.63 ± 1.26 (102.9)</td>
<td>17.59 ± 2.08 (117.1)</td>
</tr>
<tr>
<td>Sham</td>
<td>2.25 ± 0.2 (100)</td>
<td>30.50 ± 0.96 (100)</td>
<td>55.00 ± 3.14 (100)</td>
<td>15.02 ± 1.61 (100)</td>
</tr>
<tr>
<td>Control (SNL)</td>
<td>6.78 ± 0.6&lt;sup&gt;a&lt;/sup&gt; (301.4)</td>
<td>151.6 ± 14.0&lt;sup&gt;a&lt;/sup&gt; (497.0)</td>
<td>10.01 ± 0.77&lt;sup&gt;a&lt;/sup&gt; (18.19)</td>
<td>5.86 ± 0.26&lt;sup&gt;a&lt;/sup&gt; (39.05)</td>
</tr>
<tr>
<td>SNL + Res (5)</td>
<td>5.91 ± 0.7 (262.61)</td>
<td>139.3 ± 15.4 (456.9)</td>
<td>15.63 ± 1.30 (28.42)</td>
<td>7.46 ± 0.43 (49.7)</td>
</tr>
<tr>
<td>SNL + Res (10)</td>
<td>4.34 ± 0.3&lt;sup&gt;b,c&lt;/sup&gt; (193.1)</td>
<td>101.8 ± 8.2&lt;sup&gt;b,c&lt;/sup&gt; (334.1)</td>
<td>26.54 ± 2.18&lt;sup&gt;b&lt;/sup&gt; (48.25)</td>
<td>11.20 ± 0.74&lt;sup&gt;b&lt;/sup&gt; (74.60)</td>
</tr>
<tr>
<td>SNL + Res (20)</td>
<td>3.28 ± 0.3&lt;sup&gt;b,d&lt;/sup&gt; (145.6)</td>
<td>81.8 ± 7.4&lt;sup&gt;b,d&lt;/sup&gt; (268.5)</td>
<td>38.80 ± 2.70&lt;sup&gt;b&lt;/sup&gt; (70.55)</td>
<td>13.23 ± 1.53&lt;sup&gt;b&lt;/sup&gt; (88.08)</td>
</tr>
<tr>
<td>SNL + Mon (0.5)</td>
<td>6.00 ± 0.6 (266.6)</td>
<td>133.2 ± 8.3 (436.9)</td>
<td>16.82 ± 2.63 (30.58)</td>
<td>6.75 ± 0.81 (44.96)</td>
</tr>
<tr>
<td>SNL + Mon (1)</td>
<td>5.60 ± 0.58&lt;sup&gt;b&lt;/sup&gt; (248.7)</td>
<td>113.2 ± 9.0&lt;sup&gt;b,c&lt;/sup&gt; (371.4)</td>
<td>21.55 ± 1.13&lt;sup&gt;b&lt;/sup&gt; (39.18)</td>
<td>9.42 ± 0.84&lt;sup&gt;b,c&lt;/sup&gt; (62.75)</td>
</tr>
<tr>
<td>SNL + Mon (2)</td>
<td>4.14 ± 0.35&lt;sup&gt;b,e&lt;/sup&gt; (184.0)</td>
<td>95.5 ± 9.8&lt;sup&gt;b,e&lt;/sup&gt; (313.1)</td>
<td>29.26 ± 2.24&lt;sup&gt;b,e&lt;/sup&gt; (53.21)</td>
<td>11.23 ± 0.93&lt;sup&gt;b,e&lt;/sup&gt; (74.76)</td>
</tr>
<tr>
<td>SNL + Res (5) + Mon (0.5)</td>
<td>4.24 ± 0.20&lt;sup&gt;c,f&lt;/sup&gt; (188.4)</td>
<td>100.2 ± 11.1&lt;sup&gt;c,f&lt;/sup&gt; (328.6)</td>
<td>24.31 ± 2.23&lt;sup&gt;c,f&lt;/sup&gt; (44.20)</td>
<td>11.63 ± 1.32&lt;sup&gt;c,f&lt;/sup&gt; (77.45)</td>
</tr>
<tr>
<td>SNL + Res (10) + Mon (1)</td>
<td>3.37 ± 0.31&lt;sup&gt;d,e&lt;/sup&gt; (150.0)</td>
<td>77.4 ± 9.1&lt;sup&gt;d,e&lt;/sup&gt; (253.9)</td>
<td>26.66 ± 2.77&lt;sup&gt;d,e&lt;/sup&gt; (53.93)</td>
<td>12.26 ± 0.65&lt;sup&gt;d,e&lt;/sup&gt; (81.66)</td>
</tr>
</tbody>
</table>

Data is expressed as mean ± S.E.M; in parenthesis percentage of sham was mentioned. <sup>a</sup>p < 0.05 compared to sham group; <sup>b</sup>p < 0.05 compared to SNL group; <sup>c</sup>p < 0.05 compared to Res (5); <sup>d</sup>p < 0.05 compared to Res (10); <sup>e</sup>p < 0.05 compared to Mon (1); <sup>f</sup>p < 0.05 compared to Mon (0.5); (one way ANOVA followed by Tukey’s test). SNL: spinal nerve ligation; Res: resveratrol; Mon: montelukast.
Chapter 4

4.3.5. **Effect of resveratrol, montelukast and their combination on spinal cord monoamine levels in SNL treated animals**

There is no significant difference in spinal cord noradrenaline (NA) and serotonin (5-HT) levels between sham and naive group animals. SNL treatment significantly reduced NA and 5-HT levels in the lumbar spinal cord as compared to sham group. Nonetheless, resveratrol (10, 20 mg/kg) treatment significantly ($p<0.01$) restored NA and 5-HT levels (Fig. 4.5) as compared to SNL control group. Further, treatment of resveratrol (5 mg/kg), montelukast (0.5, 1 and 2 mg/kg) did not demonstrate any significant effects on spinal cord NA and 5-HT levels as compared to SNL control.

![Graph showing the effect of resveratrol and montelukast on spinal cord monoamines](image)

**Figure 4.5 Effect of resveratrol, montelukast and their combination on spinal cord noradrenalin and serotonin levels in SNL treated animals.** $^a p < 0.05$ compared to sham group; $^b p < 0.05$ compared to SNL group; (one way ANOVA followed by Tukey’s test). SNL: spinal nerve ligation; Res: resveratrol; Mon: montelukast.

However, treatment with resveratrol (5 and 10 mg/kg) in combination with montelukast (0.5 and 1 mg/kg) did not show any significant effects on spinal cord NA and 5-HT levels as compared to their effects per se in SNL treated animals. However, *per se* treatments of resveratrol (20 mg/kg) and montelukast (2 mg/kg) for 28 days did not show any significant effects on spinal cord NA and 5-HT levels as compared to sham group (data not shown).
4.4. Discussion

Ligation of L5 and L6 spinal nerve caused neuropathic pain like symptoms characterized by development of significant allodynia and hyperalgesia against different mechanical and thermal stimuli, oxidative damage, increased neuroinflammatory cytokine (TNF-α) level, as well as reduced monoamine [noradrenaline (NA) and serotonin (5-HT)] levels in lumbar spinal cord sections. Moreover, treatment with resveratrol, montelukast for 28 days significantly ameliorated these behavioural, biochemical, cellular and neuro transmitter alterations suggesting their neuroprotective potential. Further, treatment of resveratrol in combination with montelukast potentiated their therapeutic effect suggesting possible interaction between descending modulation of pain and lipoxygenase pathway in SNL induced neuropathic pain like conditions.

SNL has been well established model of NP in animals. Further, the pain behaviors that has been observed in this model persist for more than 8 weeks (Yowtak et al. 2011). In consonance with this, the present study displayed significant sensory impairment (decrease in paw withdrawal threshold to mechanical alldynic and hyperalgesic stimuli, and decreased paw licking/lifting duration to cold stimuli) suggesting the development of neuropathic pain which are persistent for 28 days. Treatment with resveratrol (a well known antioxidant) resulted in significant reversal of these behavioral alterations suggesting its therapeutic potential in neuropathic pain. Studies has suggested the neuroprotective effect of resveratrol in diverse experimental systems (Yin et al. 2013) (Kumar et al. 2007). Montelukast also showed significant reversal of the mechanical alldynia and hyperalgesia. Further, combination of resveratrol with montelukast (cysteinyll leukotriene receptor antagonist) resulted in significant reversal of these mechanical alldynia and hyperalgesia but not cold allodynia suggesting the possible interaction between these two classes of drugs in mechanical nociceptive responses. However, lack of effect on cold allodynic response could be due to various reasons. It has been shown that A fibres mediate mechanical and C fibres mediate thermal responses and combination of these drugs might have differently affected the thermal nociceptive neurons (Shir and Seltzer 1990; Simone and Kajander 1997).
Recent studies indicated that SNL results in significant elevation of reactive oxygen/nitrogen species and oxidative stress in sciatic nerves and spinal cords. Nerve injury increases the excitability of peripheral neurons which if persistent leads to central sensitisation by stimulating dorsal horn neurons and neurons in supraspinal levels. Increased NMDA activity and excitotoxicity also have been reported in neuropathic pain conditions. This increased excitability increases the intracellular calcium levels thereby increases functioning of calcium dependant nitric oxide synthase activity and NO production. Increased NO levels have been associated with an increased pain perception. Further, impaired mitochondrial functioning has been found in NP. Thus, raised NO interacts with other free radicals (super oxide) generated during mitochondrial ETC that leads to generation of peroxynitrite radicals which directly damages DNA leading to cell death. In the present study, increased oxidative stress parameters like lipid peroxidation, nitrite and reduction in antioxidant defense system like GSH, SOD have been observed in spinal cord of neuropathic rats. Further, effect of reactive oxygen species on neurotransmitter release has also been documented in different pain models (Yowtak et al. 2011; Yowtak et al. 2013). Resveratrol being a well known antioxidant has shown protective effects in SNL induced oxidative stress. Further, owing to its protective effect on ROS further, the downstream effects on neurotransmitters can’t be totally ruled out. Besides, montelukast also showed significant antioxidant effects in different neurodegenerative conditions. Recent reports from our own laboratory demonstrated the neuroprotective effects of montelukast because of its protective effect on oxidative stress and neuroinflammation. In the present study, resveratrol in combination with montelukast treatment significantly caused antioxidant like effects in spinal cord sections of neuropathic rats.

Change in descending monoamine levels (both NA and 5-HT) has been studied in NP models. Besides, descending inhibition of NA and 5-HT pathways impaired in NP and levels of these neurotransmitters reduced in spinal dorsal horn. This resulted in an impaired pain processing leading to an increased sensory deficit that is characteristic of NP. Reduced levels of these monoamines are well correlated with the observed sensory abnormalities in different neuropathic pain conditions. Further, restoration of these monoamines resulted in significant
reversal of hyperalgesia and allodynia in neuropathic rats. In the present study, decreased levels of NA and 5-HT were observed in the lumbar spinal cord sections of SNL rats implying the role of monoamine pathway in neuropathic pain.

It is now well established that administration of alpha2 adrenergic receptor antagonists, tricyclic antidepressants, and selective noradrenergic reuptake inhibitors enhanced the antinociception of opioids (Fairbanks et al. 2009) and showed promising results in clinical studies involving neuropathic patients (Li et al. 2011; Maizels and McCarberg 2005). Further, recently gabapentin which was approved by USFDA for diabetic neuropathy has been shown to enhance the descending monoamine pathways (Hayashida et al. 2007).

Further, descending NA and 5-HT has shown to mediate their actions via many targets including alteration in endogenous opioidergic transmission. Resveratrol has been shown to produce antidepressant like effects via enhancing monoamine levels in spinal and supraspinal regions in animal models. In the present study also resveratrol has been shown to increase NA and 5HT levels in neuropathic rats. This further strengthens the role of descending monoamine pathway in neuropathic pain. Besides, inhibitors of LOX products also synergised the opioid mediated inhibitory pain transmission at spinal levels (Conroy et al. 2010). So, simultaneously targeting the descending inhibitory pathway and LOX pathways could be a useful therapeutic approach in the pain management. Supporting to above, in the present study, coadministration of resveratrol with montelukast demonstrated enhanced effect as compared to their individual effect.

Lipoxygenase products such as 12/15-LO have been shown to be beneficial in preventing the development of arthritis where as 5-LO products have got pronociceptive properties based on their ability to sensitise peripheral and spinal nociceptors. Further, studies also confirmed the presence of cysteiny1 leukotriene receptors in DRG, spinal cord microglia and their contribution to pain development in rats (Okubo et al. 2010; Zhou et al. 2014). Montelukast exerted neuroprotective actions via reduction in spinal cord proinflammatory mediators (TNF-alpha, IL-6, IL-1 beta), p-p38 MAPK and NF-kB. NF-kB is a prime culprit in this inflammatory action during nerve injury. Its downstream targets like increased production of TNF-alpha, COX which play significant role in inflammatory pain
processing. Besides, antiinflammatory actions of resveratrol have also been documented (Csiszar et al. 2006; Maeba et al. 2005; Zhu et al. 2008). By taking into consideration of effects of these two drugs on inflammatory cytokines although not studied in the present study, it can't be totally overruled their possibility of antiinflammatory actions in neuropathic pain.

Further, combination of resveratrol with montelukast potentiated their effects on inflammatory cytokines production suggesting their possible positive interaction. This further strengthening the rationale for their combination in neuropathic pain. Descending monoamine pathway works in close approximation with different other systems like endogenous opioid system etc. Combinations of antidepressants and opioids have been shown to be effective in few trials but are associated with different side effect profiles. Further, leukotrienes in combination with opioids have been shown promising results. So, combing these drugs could alter descending monoamine control and leukotriene system might be useful approach. Results of the present study also provide an evidence for the rational of combing resveratrol with montelukast in neuropathic pain. However, further studies are warranted to check the exact link between these two classes of drugs in chronic pain.

4.5. Conclusion
The current study suggests the possible protective effect of resveratrol, montelukast and their combination in SNL induced behavioural, biochemical, and neurochemical alterations in rats. Study further provide a hope that targeting descending monoamine pathway and leukotriene pathways could be a useful approach in the management of SNL induced neuropathic like symptoms.