SECTION 5

RESULTS
5.1 Effect of spironolactone and telmisartan on hyperalgesia and allodynia in CCI-induced neuropathic pain

CCI resulted in significant development of cold allodynia (Figure 6), mechanical hyperalgesia (Figure 7), heat hyperalgesia (Figure 8) and mechanical allodynia (Figure 9) as compared to sham group, assessed by employing acetone, pin prick, hot plate and Von Frey tests, respectively. 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 attenuated CCI-induced hyperalgesia and allodynia in a significant manner. Administration of spironolactone (5 mg/kg, p.o.), telmisartan (1 mg/kg, p.o.) and vehicle (0.5% CMC) did not alter CCI-induced hyperalgesia and allodynia significantly. Furthermore, per se administration of spironolactone (20 mg/kg p.o), telmisartan (5 mg/kg, p.o.) and pregabalin (10 mg/kg, p.o.) did not modulate behavioral functions in normal rats.

5.2 Effect of spironolactone and telmisartan on CCI-induced spontaneous pain and foot deformation

In CCI subjected rats, the cumulative lifting (68.55 ± 8.25 s) and licking (18.45 ± 3.15 s) duration was significantly higher as compared to lifting (1.75 ± 0.65 s) and licking duration (0.55 ± 0.25 s) in sham group during the time interval of 10 minutes on 14th day. The duration of cumulative lifting (38.65 ± 5.25 s and 17.75 ± 3.15 s) and licking (14.45 ± 2.65 s and 5.65 ± 1.15 s) in spironolactone treated groups with doses of 10 and 20 mg/kg; cumulative lifting (35.15 ± 4.95 s and 16.45 ± 3.75 s) and licking (5.10 ± 0.95 s and 2.35 ± 0.70 s) in telmisartan treated groups with the doses of 2 and 5 mg/kg; the cumulative lifting (14.35 ± 3.15 s) and licking (2.05 ± 0.65 s) in pregabalin treated group with a dose of 10 mg/kg was significantly lower as compared to CCI rats.

In CCI subjected rats, a significant postural defect in terms of foot deformation was observed as compared to near normal postural index of sham group (Figure 10). 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.) significantly prevented the CCI-induced development of foot deformity. Per se administration of spironolactone (20 mg/kg p.o), telmisartan (5 mg/kg, p.o.), pregabalin (10 mg/kg, p.o.) in normal rats and vehicle (0.5 % CMC) in CCI subjected rats did not modulate behavioral functions and postural index.
5.3 Effect of spironolactone and telmisartan on hyperalgesia and allodynia in vincristine-induced neuropathic pain

Administration of vincristine (50 µg/kg, i.p.) for 10 days resulted in significant development of cold allodynia (Figure 11), mechanical hyperalgesia (Figure 12), heat hyperalgesia (Figure 13) and mechanical allodynia (Figure 14) assessed on 14th day, as compared to saline treated control group. Administration of spironolactone (5, 10 and 20 mg/kg, p.o.) and telmisartan (1, 2 and 5 mg/kg, p.o.), for 14 days, did not modulate vincristine-induced hyperalgesia and allodynia a significant manner. However, administration of pregabalin (10 mg/kg, p.o.) attenuated vincristine-induced neuropathic pain related behavioral changes. Per se administration of spironolactone (20 mg/kg p.o), telmisartan (5 mg/kg, p.o.), pregabalin (10 mg/kg, p.o.) in normal rats and vehicle (0.5 % CMC) in vincristine-injected rats did not modulate behavioral functions.

5.4 Effect of vincristine, spironolactone and telmisartan on spontaneous pain and foot deformation

Administration of vincristine (50 µg/kg, i.p.) for 10 days did not produce spontaneous pain in terms of cumulative duration of paw lifting (1.3 ± 0.6 s) and paw licking (0.2 ± 0.1 s) in 10 minutes time interval, noted on 14th day as compared to paw lifting (1.0 ± 0.4 s) and paw licking (0.2 ± 0.1 s) in corresponding saline treated rats. Administration of spironolactone (5, 10 and 20 mg/kg, p.o.), telmisartan (1, 2 and 5 mg/kg, p.o.) and pregabalin (10 mg/kg, p.o.) did not modulate duration of paw lifting and paw licking in vincristine injected rats.

Administration of vincristine (50 µg/kg, i.p.) for 10 days did not produce foot deformity and postural defect assessed on 14th day. Administration of spironolactone (5, 10 and 20 mg/kg, p.o.), telmisartan (1, 2 and 5 mg/kg, p.o.) and pregabalin (10 mg/kg, p.o.) did not modulate postural changes in vincristine injected rats. Per se administration of spironolactone (20 mg/kg p.o), telmisartan (5 mg/kg, p.o.), pregabalin (10 mg/kg, p.o.) in normal rats and vehicle (0.5 % CMC) in vincristine injected rats did not produce any effect on parameters of spontaneous pain and postural index.
5.5 Effect of spironolactone and telmisartan on TNF-α in CCI and vincristine-induced neuropathic pain

CCI resulted in significant elevation of TNF-α in the sciatic nerve on the 14\textsuperscript{th} day as compared to sham control rats. Administration of spironolactone (10 and 20 mg/kg \textit{p.o.}), telmisartan (2 and 5 mg/kg, \textit{p.o.}) and pregabalin (10 mg/kg, \textit{p.o.}), for 14 days, significantly attenuated CCI-induced increase in TNF-α levels (Figure 15). \textit{Per se} administration of spironolactone (20 mg/kg \textit{p.o.}), telmisartan (mg/kg, \textit{p.o.}), pregabalin (10 mg/kg, \textit{p.o.}) in normal rats and vehicle (0.5 % CMC) in CCI subjected rats did not produce any effect on TNF-α. Administration of vincristine (50 µg/kg, \textit{i.p.}) for 10 days did not modulate TNF-α in the sciatic nerve as compared to corresponding saline treated rats. Administration of spironolactone (5, 10 and 20 mg/kg), telmisartan (1, 2 and 5 mg/kg, \textit{p.o.}) and pregabalin (10 mg/kg, \textit{p.o.}), for 14 days, did not modulate the levels of TNF-α in vincristine injected rats (Figure 16).

5.6 Effect of FTS and GW 5074 on CCI-induced hyperalgesia and allodynia

Single intra-thecal administration of FTS (5 and 10 µg), GW 5074 (2 and 4 µg) and pregabalin (10 µg) on 14\textsuperscript{th} day of CCI subjected rats produced significant analgesic action and attenuated cold allodynia (Figure 17), mechanical hyperalgesia (Figure 18), heat hyperalgesia (Figure 19) and mechanical allodynia (Figure 20) at different time intervals \textit{i.e.}, 30 min, 60 min, 120 min and 180 min. The peak analgesic effects of FTS, GW 5074 and pregabalin were observed at 60 min after their intra-thecal administration in CCI subjected rats. However, the lower dose of FTS (2.5 µg) and GW 5074 (1 µg) did not produce any significant alterations in CCI-induced pain related changes in rats. Furthermore, \textit{per se} administration of FTS (10 µg), GW 5074 (4 µg), pregabalin (10 µg) in normal rats and DMSO (solvent) in CCI rats did not modulate pain related behavior parameters.

5.7 Effect of FTS and GW 5074 on vincristine-induced hyperalgesia and allodynia

Single intra-thecal administration of FTS (5 and 10 µg), GW 5074 (2 and 4 µg) and pregabalin (10 µg) on 14\textsuperscript{th} day in vincristine administered rats produced significant analgesic action and attenuated cold allodynia (Figure 21), mechanical hyperalgesia (Figure 22), heat hyperalgesia (Figure 23) and mechanical allodynia (Figure 24) at different time intervals \textit{i.e.}, 30 min, 60 min, 120 min and 180 min. The peak analgesic
effects of FTS, GW 5074 and pregabalin were observed at 60 min after their intra-thecal administration in vincristine administered rats with the diminished analgesic effects at 180 min. However, the lower dose of FTS (2.5 µg) and GW 5074 (1 µg) did not produce significant alterations in vincristine-induced pain related changes in rats. Furthermore, per se administration of FTS (10 µg), GW 5074 (4 µg) and pregabalin (10 µg) in normal rats and DMSO (solvent) in vincristine administered rats did not modulate pain related behavior parameters.
Figure 6: Effect of spironolactone (6a) and telmisartan (6b) on CCI-induced paw cold allodynia assessed by acetone test with pregabalin as the standard drug (Spiro: spironolactone; Telmi: telmisartan; Pregaba: pregabalin). Values are mean ± S.E.M., n=68; a= P<0.05 vs sham, b= P<0.05 vs CCI [For spironolactone, F (1, 100) = 5181.75, for days, P<0.001 and F (9, 100) = 520.45 for treatment, P<0.001; for telmisartan, F (1, 100) = 6285.15, P<0.001 for days and F (9, 100) = 580.25, P<0.001 for treatment].
Figure 7: Effect of spironolactone (7a) and telmisartan (7b) on CCI-induced mechanical hyperalgesia assessed by pin-prick test with pregabalin as the standard drug (Spiro: spironolactone; Telmi: telmisartan; Pregaba: pregabalin). Values are mean ± S.E.M., n=6; a= P<0.05 vs sham, b= P<0.05 vs CCI   [For spironolactone, F (1, 100) = 4567.25, for days, P<0.001 and F (9, 100) = 489.50 for treatment, P<0.001; for telmisartan, F (1, 100) = 5985.25, P<0.001 for days and F (9, 100) = 566.05, P<0.001 for treatment].
Figure 8: Effect of spironolactone (8a) and telmisartan (8b) on CCI-induced heat hyperalgesia assessed by hot plate test with pregabalin as standard drug (Spiro: spironolactone; Telmi: telmisartan; Pregaba: pregabalin). Values are mean ± S.E.M., n=6; a= \( P<0.05 \) vs sham, b= \( P<0.05 \) vs CCI [For spironolactone, F (1, 100) = 2357.77, for days, \( P<0.001 \) and F (9, 100) = 350.30 for treatment, \( P<0.001 \); for telmisartan, F (1, 100) = 3563.23, \( P<0.001 \) for days and F (9, 100) = 410.12, \( P<0.001 \) for treatment].
Figure 9: Effect of spironolactone (9a) and telmisartan (9b) on CCI-induced mechanical allodynia assessed by von Frey test with pregabalin as standard drug (Spiro: spironolactone; Telmi: telmisartan; Pregaba: pregabalin). Values are mean ± S.E.M., n=6; a= $P<0.05$ vs sham, b= $P<0.05$ vs CCI [For spironolactone, F (1, 100) = 3051.32, for days, $P<0.001$ and F (9, 100) = 367.29 for treatment, $P<0.001$; for telmisartan, F (1, 100) = 3815.13, $P<0.001$ for days and F (9, 100) = 440.52, $P<0.001$ for treatment].
Figure 10: Effect of spironolactone (10a) and telmisartan (10b) on CCI-induced foot deformity with pregabalin as standard drug (Spiro: spironolactone; Telmi: telmisartan; Pregaba: pregabalin). Values are mean ± S.E.M., n=8 rats per group; a= $P<$0.05 vs sham, b= $P<$0.05 vs CCI.
Cold Allodynia

**Figure 11a**

![Cold Allodynia Graph](image1)

**Figure 11b**

![Cold Allodynia Graph](image2)

**Figure 11**: Effect of spironolactone (11a) and telmisartan (11b) on vincristine-induced paw cold allodynia assessed by acetone test (Spiro: spironolactone; Telmi: telmisartan; Pregaba: pregabalin; Vin: vincristine). Values are mean ± S.E.M., n=6 a= P<0.05 vs saline control; b= P<0.05 vs vin control [For spironolactone, F (1, 100) = 1261.22, for days, P<0.001 and F (9, 100) = 102.20 for treatment, P<0.001; for telmisartan, F (1, 100) = 1782.12, P<0.001 for days and F (9, 100) = 121.52, P<0.001 for treatment].
Figure 12: Effect of spironolactone (12a) and telmisartan (12b) on vincristine-induced mechanical hyperalgesia assessed by pin-prick test (Spiro: spironolactone; Telmi: telmisartan; Pregaba: pregabalin; Vin: vincristine). Values are mean ± S.E.M., n=6; a= \( P<0.05 \) vs saline control; b= \( P<0.05 \) vs vincristine control [For spironolactone, F (1, 100) = 989.02, for days, \( P<0.001 \) and F (9, 100) = 98.12 for treatment, \( P<0.001 \); for telmisartan, F (1, 100) = 1123.19, \( P<0.001 \) for days and F (9, 100) = 104.55, \( P<0.001 \) for treatment].
Figure 13: Effect of spironolactone (13a) and telmisartan (13b) on vincristine-induced heat hyperalgesia assessed by hot plate test (Spiro: spironolactone; Telmi: telmisartan; Pregaba: pregabalin; Vin: vincristine). Values are mean ± S.E.M., n=6; a = P<0.05 vs saline control; b = P<0.05 vs vincristine control [For spironolactone, F (1, 100) = 567.12, for days, P<0.001 and F (9, 100) = 83.42 for treatment, P<0.001; for telmisartan, F (1, 100) = 980.97, P<0.001 for days and F (9, 100) = 87.25, P<0.001 for treatment].
Figure 14: Effect of spironolactone (14a) and telmisartan (14b) on vincristine-induced mechanical allodynia assessed by von Frey test (Spiro: spironolactone; Telmi: telmisartan; Pregaba: pregabalin; Vin: vincristine). Values are mean ± S.E.M., n=6; a = P<0.05 vs saline control, b = P<0.05 vs vincristine control [For spironolactone, F (1, 100) = 892.42, for days, P<0.001 and F (9, 100) = 103.44 for treatment, P<0.001; for telmisartan, F (1, 100) = 1230.71, P<0.001 for days and F (9, 100) = 121.15, P<0.001 for treatment].
Figure 15: Effect of spironolactone (15a) and telmisartan (15b) on CCI-induced changes in TNF-α in the sciatic nerve (Spiro: spironolactone; Telmi: telmisartan; Pregaba: pregabalin; Vin: vincristine). Values are mean ± S.E.M., n=6; a= P<0.05 vs sham, b= P<0.05 vs CCI [For spironolactone, F (9, 50) = 8.85, P<0.001; for telmisartan, F (9,50) = 9.12, P<0.001].
Figure 16: Effect of spironolactone (16a) and telmisartan (16b) on TNF-α levels in the sciatic nerve in vincristine-injected rats (Spiro: spironolactone; Telmi: telmisartan; Pregaba: pregabalin; Vin: vincristine). Values are mean ± S.E.M., n=6 [For spironolactone, F (9, 50) = 1.25, P=0.101; for telmisartan, F (9, 50) = 1.28, P=0.112.]
Figure 17: Effect of single intra-thecal administration of FTS (17a) and GW 5074 (17b) in CCI-induced cold allodynia assessed by acetone test on 14th day at different time intervals with pregabalin as standard drug. Values are in mean ± S.E.M., n=6; a= P<0.05 vs sham, b= P<0.05 vs CCI [For FTS, F (3, 200) = 231.22, P<0.001 for time and F (9, 200) = 6784.44 for pharmacological interventions, P<0.001; for GW 5074, F (3, 200) = 189.73, P<0.001 for time and F (9, 200) = 5668.75, P<0.001 for pharmacological interventions].
Figure 18: Effect of single intra-thecal administration of FTS (18a) and GW 5074 (18b) in CCI-induced mechanical hyperalgesia assessed by pin-prick test on 14th day at different time intervals with pregabalin as standard drug. Values are in mean ± S.E.M., n=6; a = P<0.05 vs sham, b = P<0.05 vs CCI [For FTS, F (3, 200) = 212.34, P<0.001 for time and F (9, 200) = 5794.49 for pharmacological interventions, P<0.001; for GW 5074, F (3, 200) = 178.33, P<0.001 for time and F (9, 200) = 5328.95, P<0.001 for pharmacological interventions].
Figure 19: Effect of single intra-thecal administration of FTS (19a) and GW 5074 (19b) in CCI-induced heat hyperalgesia assessed by hot plate test on 14th day at different time intervals with pregabalin as standard drug. Values are in mean ± S.E.M., n=6; a= P<0.05 vs sham, b= P<0.05 vs CCI [For FTS, F (3, 200) = 202.14, P<0.001 for time and F (9, 200) = 4894.59 for pharmacological interventions, P<0.001; for GW 5074, F (3, 200) = 178.13, P<0.001 for time and F (9, 200) = 4546.44, P<0.001 for pharmacological interventions].
Figure 20: Effect of single intra-thecal administration of FTS (20a) and GW 5074 (20b) in CCI-induced mechanical allodynia assessed by von Frey test on 14th day at different time intervals with pregabalin as standard drug. Values are in mean ± S.E.M., n=6; a= P<0.05 vs sham, b= P<0.05 vs CCI [For FTS, F (3, 200) = 154.34, P<0.001 for time and F (9, 200) = 3894.80 for pharmacological interventions, P<0.001; for GW 5074, F (3, 200) = 141.23, P<0.001 for time and F (9, 200) = 3521.14, P<0.001 for pharmacological interventions].
Figure 21: Effect of single intra-thecal administration of FTS (21a) and GW 5074 (21b) in vincristine-induced cold allodynia assessed by acetone test on 14th day at different time intervals with pregabalin as standard drug. Values are in mean ± S.E.M., n=6; a= $P<0.05$ vs saline control, b= $P<0.05$ vs vincristine [For FTS, F (3, 200) = 138.14, $P<0.001$ for time and F (9, 200) = 2341.18 for pharmacological interventions, $P<0.001$; for GW 5074, F (3, 200) = 121.13, $P<0.001$ for time and F (9, 200) = 2001.27, $P<0.001$ for pharmacological interventions].
Figure 22: Effect of single intra-thecal administration of FTS (22a) and GW 5074 (22b) in vincristine-induced mechanical hyperalgesia assessed by pin prick test on 14th day at different time intervals with pregabalin as standard drug. Values are in mean ± S.E.M., n=6; a = P<0.05 vs saline control, b = P<0.05 vs vincristine [For FTS, F (3, 200) = 120.27, P<0.001 for time and F (9, 200) = 1892.01 for pharmacological interventions, P<0.001; for GW 5074, F (3, 200) = 107.23, P<0.001 for time and F (9, 200) = 1788.17, P<0.001 for pharmacological interventions].
Figure 23a: Effect of single intra-thecal administration of FTS (23a) and GW 5074 (23b) in vincristine-induced heat hyperalgesia assessed by hot plate test on 14th day at different time intervals with pregabalin as standard drug. Values are in mean ± S.E.M., n=6; a = P<0.05 vs saline control, b = P<0.05 vs vincristine [For FTS, F (3, 200) = 112.13, P<0.001 for time and F (9, 200) = 1542.01 for pharmacological interventions, P<0.001; for GW 5074, F (3, 200) = 94.13, P<0.001 for time and F (9, 200) = 1418.15, P<0.001 for pharmacological interventions].
**Figure 24**: Effect of single intra-thecal administration of FTS (24a) and GW 5074 (24b) on vincristine-induced mechanical allodynia assessed by von Frey test on 14th day at different time intervals with pregabalin as standard drug. Values are in mean ± S.E.M., n=6; a = P<0.05 vs saline control, b = P<0.05 vs vincristine [For FTS, F (3, 200) = 112.03, P<0.001 for time and F (9, 200) = 1312.01 for pharmacological interventions, P<0.001; for GW 5074, F (3, 200) = 89.23, P<0.001 for time and F (9, 200) = 1213.55, P<0.001 for pharmacological interventions].