CHAPTER IV
RESULTS

1. Assessment of general toxicity

Two rats died, in the V and P group, after the third and fourth injections, respectively. All other animals survived until the end of the study and received five injections as scheduled in the protocol. The body weights of paclitaxel-treated rats (P group) started to significantly decrease compared to those of the other groups at the first week of treatment and remained lower until the end of the experiment.

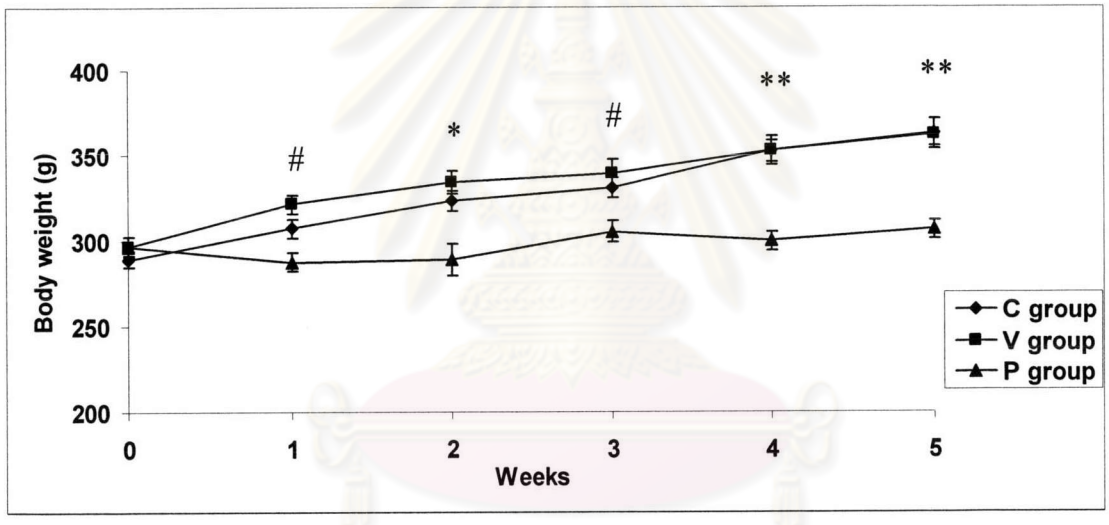

Figure 5 Body weights (g) of rats in the control, vehicle and paclitaxel groups during the experiment. Data are expressed in means and error bars represent SEM.
C = Control, V = Vehicle, P = Paclitaxel
(# $p < 0.05$, * $p < 0.01$, ** $p < 0.001$, P compared to C and V groups)

2. Quantitative sensory tests

2.1 Tail flick analgesic test

Reaction time of the rats in the three experimental groups in response to heat at the tail is demonstrated in Figure 6. At baseline, there were no significant differences between groups in the average heat-evoked response reaction times. This value was significantly prolonged in the P group relative to the others in week 1-2 ($p < 0.05$) and relative to the C group in week 3 ($p < 0.05$) and returned to the similar level seen in other groups in the last two weeks of the experiment. In V group, reaction time was significantly prolonged relative to the C group in week 3 ($p < 0.05$).

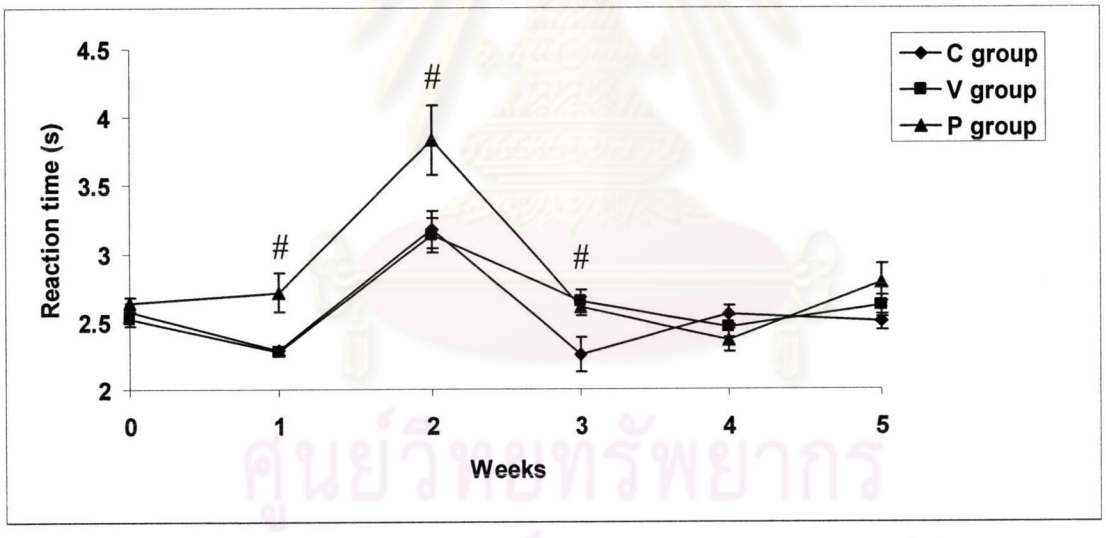

Figure 6 Reaction time from tail flick analgesic test of control, vehicle and paclitaxel groups. Data are expressed in means and error bars represent SEM.
C = Control, V = Vehicle, P = Paclitaxel
(# $p < 0.05$, P compared to C and V groups)

2.2 Hind paw analgesic test

At baseline (Figure 7), there were no significant differences between groups in the average heat-evoked response reaction times. In paclitaxel-injected rats, compared to control ones, a significant increase in the hind paw withdrawal time in the third week was observed ($p < 0.05$).

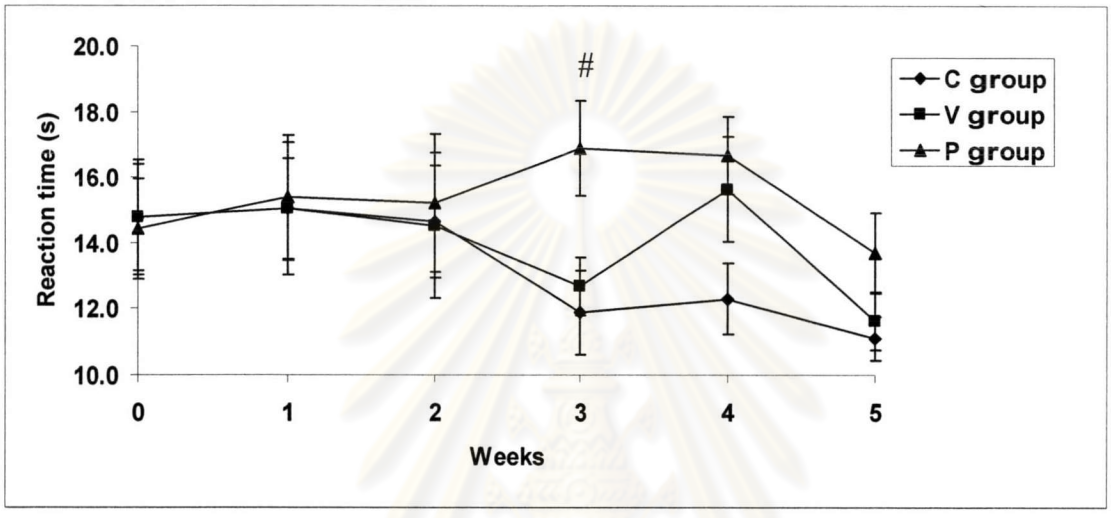

Figure 7 Reaction time from hind paw analgesic test of control, vehicle and paclitaxel groups. Data are expressed in means and error bars represent SEM.
C = Control, V = Vehicle, P = Paclitaxel
(# $p < 0.05$, P vs. C groups)

3. Electrophysiological measurement

No difference between groups was observed at baseline determination (data not shown). The administration of paclitaxel induced a significant reduction in tail nerve conduction velocity compared with the control group in the second week ($p < 0.01$) and fifth week ($p < 0.05$). Tail nerve conduction velocity was not significantly different among the experimental groups in other time-points.

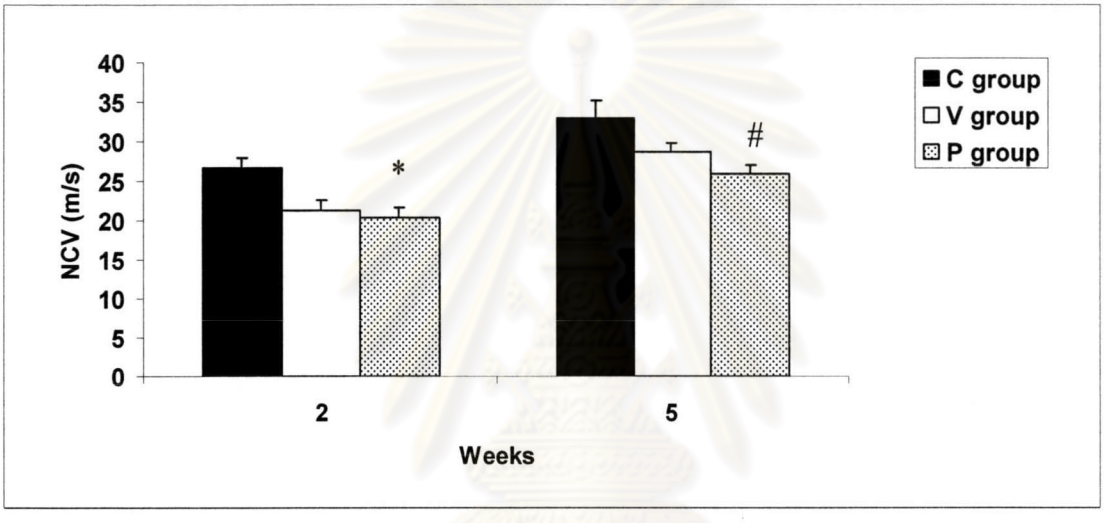

Figure 8 Tail nerve conduction velocity (NCV) of control, vehicle and paclitaxel groups. Data are expressed in means and error bars represent SEM.
C = Control, V = Vehicle, P = Paclitaxel
(# $p < 0.05$ (P vs. C groups), * $p < 0.01$, C compared to V and P groups)

4. Phosphorylation of MAPKs

DRG from the rats with paclitaxel-induced neuropathy were used to study the phosphorylation of MAPKs along with those from the control and vehicle-treated groups. Activation of MAPK pathways was examined using Western blotting. The antibodies raised against phosphorylated epitopes of the kinases and antibodies raised against the kinases irrespective of their phosphorylation states were used to detect the proteins. A ratio of phosphorylated to total proteins was then determined and changes in the phosphorylation states were analyzed.

4.1 ERK phosphorylation

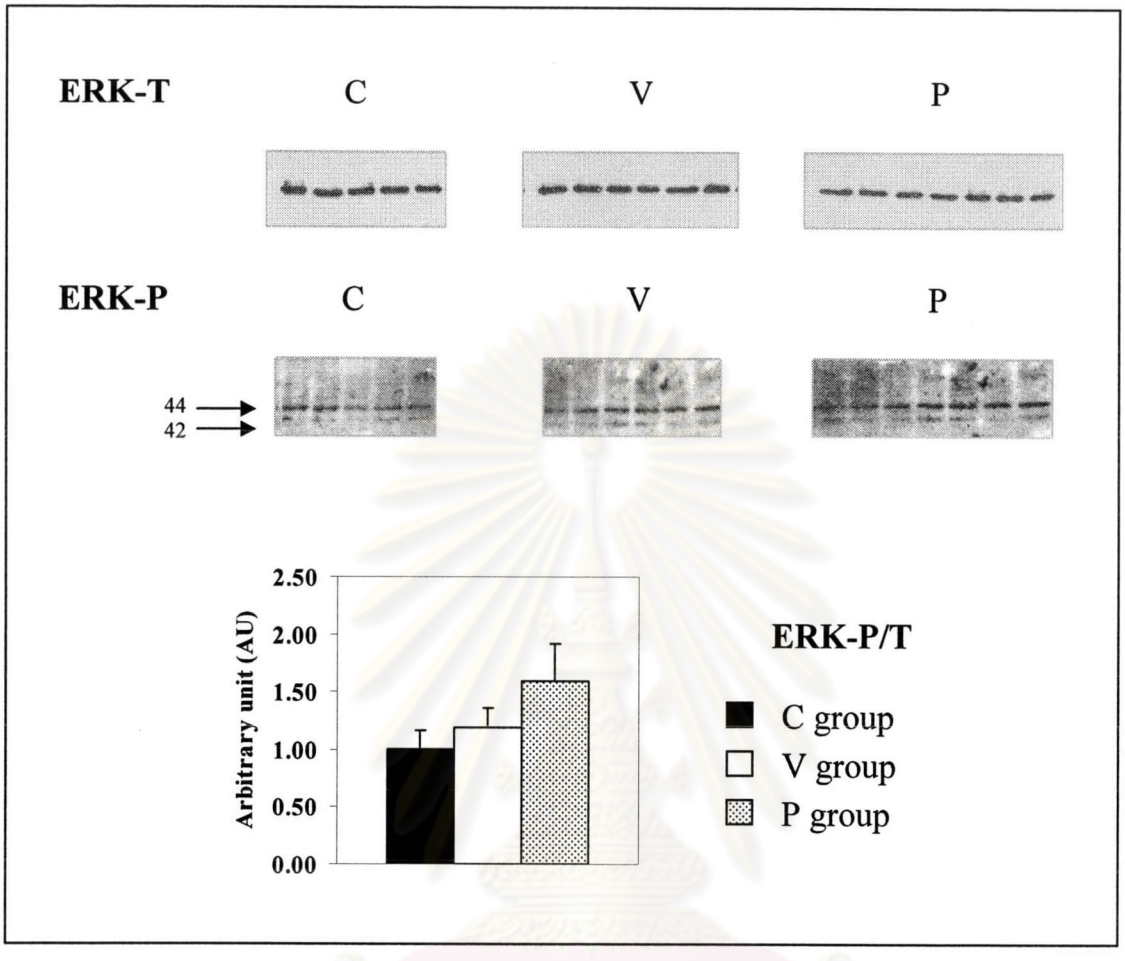

Figure 9 ERK phosphorylation in $L_{4/5}$ DRG from control, vehicle and paclitaxel groups at the end of the experiment.
The immunoblots show the levels of phospho-ERK (ERK-P) and total-ERK (ERK-T). Arrows indicate the two isoforms of ERK, ERK1 (44 kDa) and ERK (42 kDa). The graph shows the ratio of ERK-P to ERK-T for both isoforms in combination. Data are expressed as means $\pm$ SEM. (C = Control, V = Vehicle, P = Paclitaxel)

Results from Western blot analysis showed that ERK phosphorylation was slightly increased in the P group relative to the other groups although the change was statistically insignificant (Figure 9). There were no differences in the levels of ERK-T among the three groups.

4.2 JNK phosphorylation

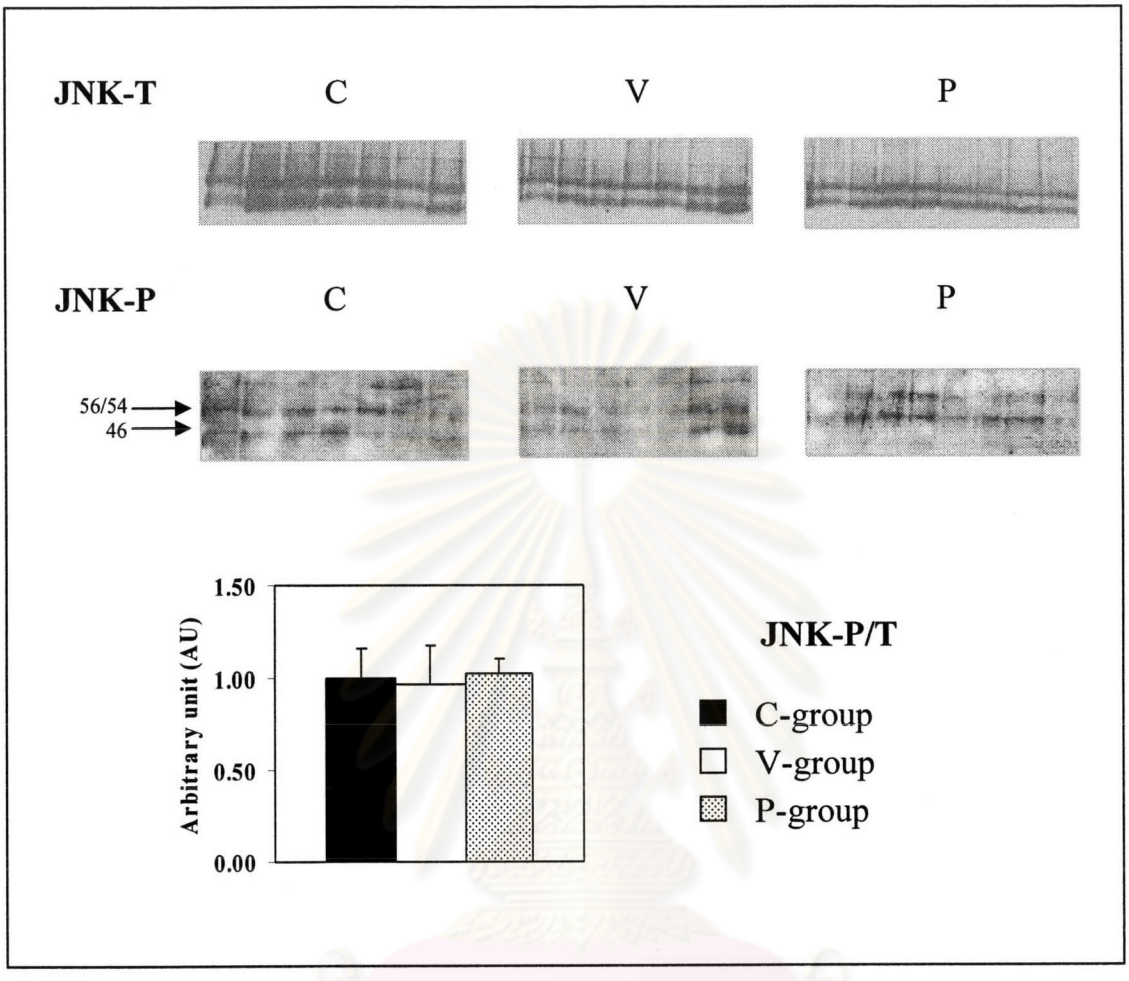

Figure 10 JNK phosphorylation in $L_{4/5}$ DRG from control, vehicle and paclitaxel groups at the end of the experiment.
The immunoblots show the levels of phospho-JNK (JNK-P) and total-JNK (JNK-T). Upper and lower arrows indicate two bands of JNK at 56/54 kDa and 46 kDa, respectively and the graph shows the ratio of JNK-P to JNK-T. Data are expressed as means $\pm$ SEM. (C = Control, V = Vehicle, P = Paclitaxel)

Regarding JNK, the levels of JNK-T were similar in all groups. Furthermore, JNK phosphorylation was not different among the three groups (Figure 10).

4.3 p38 phosphorylation

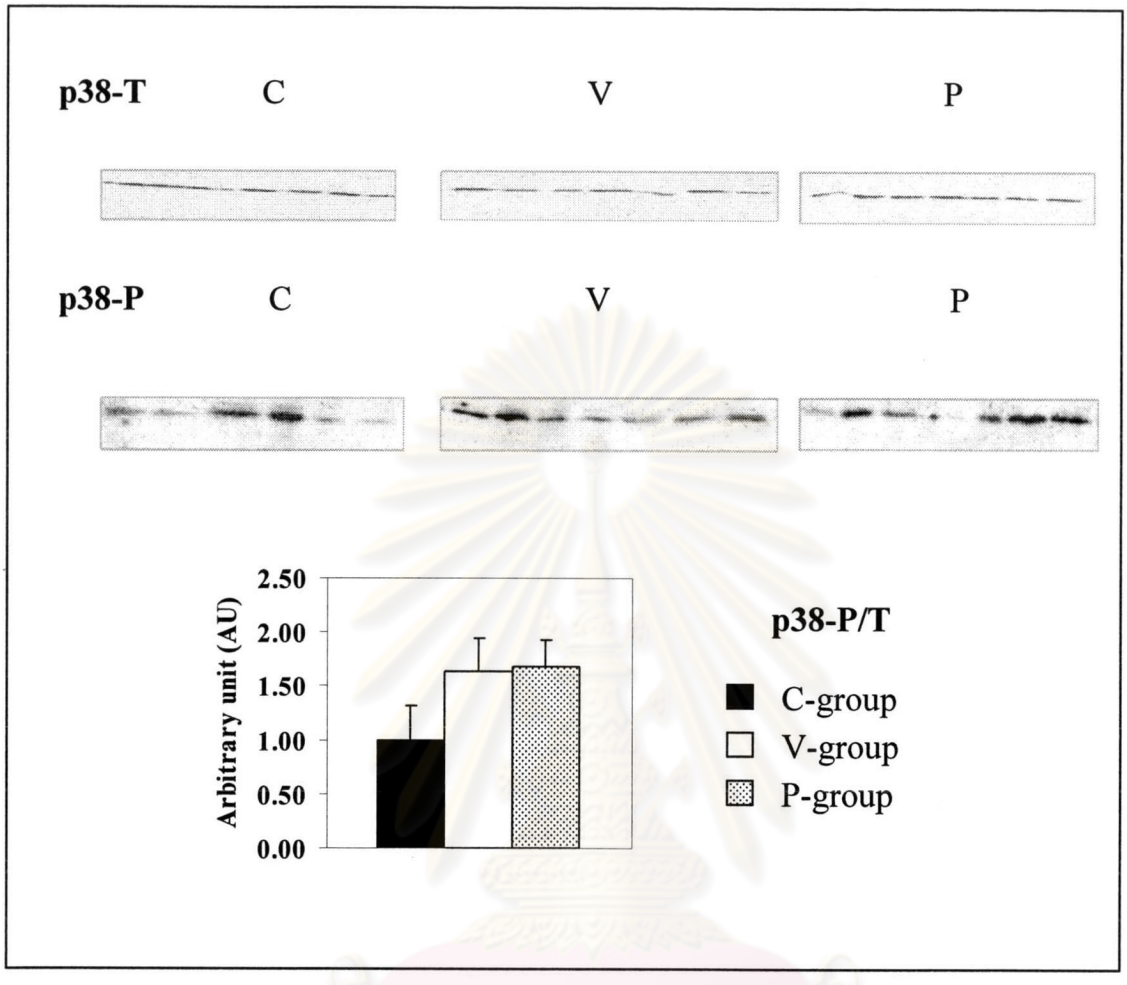

Figure 11 p38 phosphorylation in $L_{4/5}$ DRG from control, vehicle and paclitaxel groups at the end of the experiment.
The immunoblots show the levels of phospho-p38 (p38-P) and total-p38 (p38-T). The graph shows the ratio of p38-P to p38-T. Data are expressed as means ± SEM. (C = Control, V = Vehicle, P = Paclitaxel)

An insignificant increase in p38 phosphorylation was observed in the P and V groups after 5 weeks of treatment (Figure 11). There was no significant difference in the levels of p38-T among the experimental groups.

In conclusion, increased thermal threshold was observed at the tail and hind paw of the paclitaxel-treated rats versus rats from the other groups during the experiment. Morever, tail nerve conduction velocity was also reduced in the P group compared to the other groups at week 2 and week 5. According to the results from Western blot analysis, ERK and p38 were slightly activated in the P group but this was not statistically significant compared to the C group at the end of the experiment. It is worth nothing that p38 phosphorylation in the V group was also slightly elevated similar to the P group.