CHAPTER V

DISCUSSION & CONCLUSION

These studies have demonstrated the activity of the *Cissus quadrangularis* dried stems extract (CQ) to generate significant analgesia in different animal models including hot-plate, tail-flick and paw-pressure tests. The hot-plate response and the paw-pressure response is believed to be a more complex supraspinally organized behavior and the tail-flick response is a spinally mediated reflex (Chapman, et al., 1985). Initial attempts to investigate the analgesic effect of CQ utilized the standard mouse hot-plate technique. CQ doses of 43.5-700 mg/kg demonstrated a dose-response relationship with the mouse hot-plate technique (Figure 4-6). The linear regression equation for all doses of CQ was [Response = 4063.6035*LOG(X) - 3382.7760], r^2= 0.82 and [Response = 5871.9963*LOG(X) - 6907.8983]. r^2 = 0.97 when excluding the highest dose of CQ (Figure 4-8 & 4-9). The analgesic action of CQ was observed during 240 min period (Figure 4-6). Morphine (MO) as a reference standard had shown potent analgesic effect but acetylsalicylic acid had little influence on the response in this animal model. CQ doses of 350 and higher appeared to produce higher analgesic response compared to ASA and CQ 350 mg/kg produced analgesic response similar to MO (Figure 4-7). The ED_{50} of CQ was equal to 440.62 (44.28-4384.77) mg/kg (Figure 4-10).

NAL and NALT, opioid receptor antagonists, were utilized to investigate the involvement of opioid receptor in the analgesic effects of CQ (Figure 4-12 & 4-13). The results from NALT showed the involvement of opioid receptor in analgesia produced by CQ; while NAL showed no effect possibly because of its shorter half-life (Figure 4-12). Since NMDA coadministration did not appeared to attenuate the analgesic response of CQ suggested no involvement of NMDA receptor in CQ analgesia (Figure 4-14).

Studies were then undertaken to investigate the effectiveness of CQ utilizing the mouse tail-flick technique. MO administered i.p. produced significant analgesic response as expected (Figure 4-15), ASA also produced analgesia but at a lesser extent compared to

MO in this animal model (Figure 4-16). The lowest dose of CQ (43.5 mg/kg) had no analgesic effect in this model (Figure 4-17). CQ at doses of 87.5-700 mg/kg administered i.p. produced a dose-related analgesic response (Figure 4-17). The linear regression equation for all doses of CQ was [Response = 4628.1721*LOG(X) - 7689.7635], r^2= 0.93 (Figure 4-19).

The Randall Selitto paw-pressure test was chosen to measure CQ effect against mechanical stimuli. Intraperitoneal administration of MO and ASA at dose tested produce significant analgesic responses compared to NSS treated controls utilizing this method (Figure 4-21 & 4-22). Similar to mouse hot-plate and tail-flick tests, CQ demonstrated a dose-response relationship with the rat paw-pressure technique (Figure 4-23). The linear regression equation of all doses of CQ was [Response = 1649.8514*LOG(X) - 2024.0220], r^2= 0.99 (Figure 4-25).

We also examined the effect of CQ on the inflamed paw pressure test. This model compared withdrawal threshold observed from an intact paw with an inflamed paw. The inflammation was induced by a subcutaneous injection of carrageenan into the subplantar region of rat's hind paw. This inflamed paw-pressure response is considered as a hyperalgesia pain model (Hargreaves, K., 1988; Megaraughty et al., 2001). Intraperitoneal administration of MO and IND at dose tested produce similar analgesic response compared to NSS treated controls utilizing this method (Figure 4-27&4-28). CQ doses of 350 mg/kg and higher produced analgesic responses compared to NSS treated controls (Figure 4-29). All doses of CQ failed to inhibit carrageenan-induced hyperalgesia compared to NSS treated controls (Figure 4-30). The linear regression equation of all doses of CQ was [Response = 172.6908*LOG(X) - 245.9029], r^2= 0.98 (Figure 4-31).

CQ has demonstrated analgesic response in all testing models suggesting that CQ could produce analgesia via both spinal and supraspinal mechanisms. The analgesic peak effect of CQ was reached within 60-90 minutes after i.p. administration. The hot-plate analgesia testing seemed to be the most sensitive test for evaluating analgesic effect of CQ as all doses of CQ had shown higher analgesic response compared to NSS treated group.

In conclusion, the current study has demonstrated that as reported for many naturally-occurring substances isolated from plants, the ethanolic fraction obtained from *Cissus quadrangularis* dried stems exerts a pronounced antinociception when assessed in thermal and mechanical models of nociception in rodents, these effects being due, at least in part, to the presence of triterpene and phytosterol compounds. The precise mechanism involved in their action is, at this moment, not completely understood; it is most likely involved with the opioid pathway.

FUTURE RESEARCH

In these studies, there was evidence that the ethanolic extract of *Cissus quadrangularis* dried stems was capable of significantly produced analgesic response, most likely via an opioid-mediated mechanism, and supports the potential use of the extract.

The future research could comprise of several objectives as listed below:

(1) To identify the active components of the extract.
(2) To investigate the antipyretic effect of various doses of CQ.
(3) To investigate the anesthetic effect of various doses of CQ.
(4) To investigate the potential use of CQ in combination with other analgesic or nonsteroidal anti-inflammatory drugs.
(5) To investigate other routes of administration that might be more appropriate for the use of CQ and possibly enhance the analgesic effect of CQ.
(6) To better understand the mechanism of CQ that is involved in producing its analgesic effect.
(7) To test side effects and toxic effects of CQ at high doses.

These and other studies may provide important clues to help understand the mechanisms underlying the analgesic effect of CQ and further support the use of such compounds in a clinical setting.