CHAPTER 3

RESULTS

Part I. Anti-inflammatory and analgesic activity of the methanol extract from *C. serratum* Linn.

3.1 Anti-inflammatory activity of the CS extract

3.1.1 Effects of the CS extract, diclofenac and Daflon® on EPP-induced rat ear edema

The inhibitory effect produced by the topical administration of the CS extract on EPP-induced ear edema was assessed. As shown in Table 2, the ear edema thicknesses of the control group (received acetone), were found to be 106.67 ± 4.94, 170 ± 8.16, 180 ± 5.77 and 110 ± 10.00 μm at 15, 30, 60 and 120 min after EPP application, respectively. Diclofenac, a potent NSAID, at the dose of 5 mg/ear produced significant inhibitory activity on edema formation at all determination times. Daflon® at the dose of 4 mg/ear also exhibited significant inhibitory effect on EPP-induced ear edema at 30, 60 and 120 min after EPP application. At the dose of 2 mg/ear, the CS extract significantly exerted inhibitory effect on EPP-induced ear edema at all assessment times. The results in Table 2 shows that the CS extract at the dose of 2 mg/kg produced marked anti-edema activity comparable to diclofenac at the dose of 5 mg/kg and higher than Daflon® at the dose of 4 mg/kg.

Table 2. Effects of the CS extract, diclofenac and Daflon® on EPP-induced ear edema in rats.

Group	Dose (mg/ear)	Edema thickness (μm)				% Inhibition			
		15 min	30 min	60 min	120 min	15 min	30 min	60 min	120 min
Control	-	106.67 ± 4.94	170.00 ± 8.16	180.00 ± 5.77	110.00 ± 10.00	-	-	-	-
Diclofenac	5	43.33 ± 6.15***	88.33 ± 7.03***	68.33 ± 13.52***	25.00 ± 6.71***	59	48	62	77
Daflon®	4	96.67 ± 5.58	131.67 ± 8.72***	95.00 ± 11.76***	30.00 ± 6.32***	9	23	47	73
CS extract	2	53.33 ± 8.43***	105.00 ± 6.19***	91.67 ± 10.77***	18.33 ± 3.07***	50	38	49	83

Test drugs were applied topically to both inner and outer surfaces of the ear. The control group received vehicle (acetone) only. Values represent the mean ± S.E.M. (N = 6). Significantly different from the control: $^{***}P < 0.001$.

3.1.2 Effects of the CS extract, diclofenac and Daflon® on carrageenin-induced hind paw edema in rats.

The inhibitory activities on carrageenin-induced rat hind paw edema caused by oral administration of the CS extract, diclofenac and Daflon®, at various time points after carrageenin injection are shown in Table 3. In control group (received NSS), the paw edema volumes were found to be 0.27 $\pm$ 0.02, 0.51 $\pm$ 0.04 and 0.50 $\pm$ 0.03 mL at the 1st, 3 rd and 5th h after carrageenin injection, respectively.

Diclofenac, at the dose of 10 mg/kg body weight exhibited marked edema inhibition of 70% at the 3rd h after carrageenin injection, whereas Daflon® at the dose of 300 mg/kg also exhibited significant edema inhibition of 46%. The CS extract at dose of 40 mg/kg exhibited slight but significant inhibition on carrageenin-induced hind paw edema. However, at doses of 80 and 160 mg/kg the CS extract elicited significant inhibitory effect on carrageenin-induced hind paw edema at all assessment times. The anti-edematous effect of the CS extract gradually increased as the doses increased. At the 3th h after carrageenin injection, the CS extract at doses of 80 and 160 mg/kg showed 40% and 63% edema inhibition, respectively and its inhibitory effect lasted to the 5th h after carrageenin injection.

Table 3. Effects of the CS extract, diclofenac and Daflon® on carrageenin-induced hind paw edema in rats.

Group	Dose (mg/kg)	Paw volume induced (mL)			% Inhibition		
		1h	3h	5h	1h	3h	5h
Control	-	0.27 ± 0.02	0.51 ± 0.04	0.50± 0.03	-	-	-
Diclofenac	10	0.08± 0.01***	0.15 ± 0.01***	0.23± 0.03***	72	70	55
Daflon®	300	0.14 ± 0.02***	0.28 ± 0.03***	0.29 ± 0.04***	48	46	42
CS extract	40	0.24 ± 0.02	0.43 ± 0.03	0.42 ± 0.03	11	15	17
CS extract	80	0.17 ±0.01***	0.30 ± 0.04***	0.29 ± 0.05***	37	40	43
CS extract	160	0.13 ± 0.01***	0.19 ±0.01***	0.14 ± 0.01***	52	63	72

Test drugs were orally administered 1 h before carrageenin injection. The control group received vehicle (NSS) only.

Values represent the mean ± S.E.M. (N = 6). Significantly different from the control: $^{***}P < 0.001$.

3.1.3 Effects of the CS extract, diclofenac, prednisolone and Daflon® on AA-induced hind paw edema in rats.

The injection of 0.5% AA into the plantar side of the right hind paw significantly produced edema formation by 1 h after challenge. The results obtained are shown in Table 4. One hour after AA injection, the average edema volume in the control group amounted to 0.52 ± 0.03 mL. Diclofenac at the dose of 10 mg/kg did not show any inhibitory effect on AA-induced paw edema. By contrast, prednisolone, a phospholipase A_2 inhibitor, at the dose of 5 mg/kg exhibited significant inhibitory activity on the edema of 67% when assessment was done 1 h after AA injection. Daflon® at the dose of 300 mg/kg and the CS extract at the dose of 40 mg/kg exhibited only slight and nonsignificant inhibition on the edema formation induced by AA. However, the CS extract at doses of 80 and 160 mg/kg exhibited significant reduction of paw edema with the percentages of inhibition of 37% and 51%, respectively.

Table 4. Effects of the CS extract, diclofenac, prednisolone and Daflon® on AA-induced hind paw edema in rats.

Group	Dose (mg/kg)	Edema volume (mL)	% Edema inhibition
Control	-	0.52 ± 0.03	-
Diclofenac	10	0.43 ± 0.02	8
Prednisolone	5	0.17 ± 0.04***	67
Daflon®	300	0.39 ± 0.04	26
CS extract	40	0.45 ± 0.04	14
CS extract	80	0.33 ± 0.04**	37
CS extract	160	0.26 ± 0.04***	51

Test drugs were orally administered 2 h before AA injection. The control group received vehicle (NSS) only. Values represent the mean ± S.E.M. (N = 6). Significantly different from the control: $^{**}P < 0.01$, $^{***}P < 0.001$.

3.2 Analgesic activity of the CS extract.

3.2.1 Effects of the CS extract, diclofenac, morphine and Daflon® on the formalin test in mice.

The analgesic activity of test drugs in formalin-induced pain at the right dorsal hind paw of mice was investigated both on the early phase and the late phase using the intensive licking time as a criterion for algesia.

3.2.1.1 The early phase

The results in Table 5 show that the injection of 1% formalin into the dorsal hind paw of mice produced intensive licking at the injected site with marked licking time as seen with the control group (107.83 $\pm$ 5.63 sec). Morphine at the dose of 10 mg/kg caused marked analgesic activity by abolishing the licking time. Daflon® at a dose of 150 mg/kg reduced the licking time with inhibition of 41%. Diclofenac at the dose of 5 mg/kg and the CS extract at the dose of 20 mg/kg did not show significant inhibitory effect on the period of time the mice spent in paw licking. At doses of 40 and 80 mg/kg, the CS extract significantly decreased the licking time, when compared with that of the control group with the inhibition of 42% and 48%, respectively. Both doses of the CS extract produced equivalent inhibitory effect on the time of licking response as Daflon® at a dose of 150 mg/kg.

3.2.1.2 The late phase

Inhibition of licking response of the test drugs on the late phase of the formalin test is shown in Table 6. Assessment of analgesic effect in the late phase was performed 20 min after injection of 1% formalin in to the dorsal side of hind paw of mice. The control group showed marked licking time of 145.17 ± 4.24 sec. Morphine at the dose of 10 mg/kg and diclofenac at the dose of 5 mg/kg produced marked analgesic effect by reducing the licking time caused by formalin injection with the inhibition of 100% and 94%, respectively. The CS extract at doses of 20, 40 and 80 mg/kg markedly decreased the licking time to 54.00 ± 3.30, 26.83 ± 2.93 and 8.17 ± 2.44 sec, with the inhibition of 63%, 82% and 94%, respectively. The reduction of the licking time was increased with the increased doses. At a dose of 80

mg/kg, the CS extract could produce the same inhibitory effect on the time of licking response as diclofenac at a dose of 5 mg/kg.

Table 5. Effects of the CS extract, morphine, diclofenac and Daflon® on the early phase of formalin test.

Group	Dose (mg/kg)	Licking time (sec)	% Inhibition of licking response
Control	-	107.83 ± 5.63	-
Morphine	10	0.00 ± 0.00***	100
Diclofenac	5	99.67 ± 2.67	8
Daflon®	150	63.83 ± 2.68***	41
CS extract	20	103.00 ± 6.30	4
CS extract	40	62.33 ± 1.96***	42
CS extract	80	56.00 ± 4.33***	48

Test drugs were intraperitoneally administered 1 h before formalin injection. The control group received vehicle (NSS) only. Values represent the mean ± S.E.M. (N = 6). Significantly different from the control: $^{***}P < 0.001$.

Table 6. Effects of the CS extract, morphine, diclofenac and Daflon® on the late phase of formalin test.

Group	Dose (mg/kg)	Licking time (sec)	% Inhibition of licking response
Control	-	145.17 ± 4.24	-
Morphine	10	0.00 ± 0.00***	100
Diclofenac	5	8.33 ± 1.45***	94
Daflon®	150	48.17 ± 4.08***	67
CS extract	20	54.00 ± 3.30***	63
CS extract	40	26.83 ± 2.93***	82
CS extract	80	8.17 ± 2.44***	94

Test drugs were intraperitoneally administered 40 min before formalin injection. The control group received vehicle (NSS) only. Values represent the mean ± S.E.M. (N = 6). Significantly different from the control: $^{***}P < 0.001$.

Part II

3.3 Study of the vascular effect of the methanol extract from *C. serratum* Linn. using isolated human umbilical vein.

The results show in Figures 9, 10, and 11 were the constrictive effect of test drugs on the smooth muscle of human umbilical vein. NE was used as a standard venoconstrictive. At the maximum dose of 15 μM, NE produced contraction of the vein with the intensity of 2.5 g which was assumed to be 100% contraction.

The CS extract when added to the tissue bath at doses of 0.1, 0.2, and 0.4 mg/mL bath volume produced contraction of the smooth muscle of human umbilical vein with the intensity of 0.71 ± 0.04, 1.40 ± 0.08, and 2.42 ± 0.04 g, respectively. The reference drug, Daflon® at the doses of either 0.1, 0.2 and 0.4 mg/mL bath volume similarly produced contraction of the vein with intensity of 0.52 ± 0.02, 1.04 ± 0.09, and 2.31 ± 0.07 g, respectively. Figure 8 shows percent contraction of the CS extract and Daflon® as compared to that of NE.

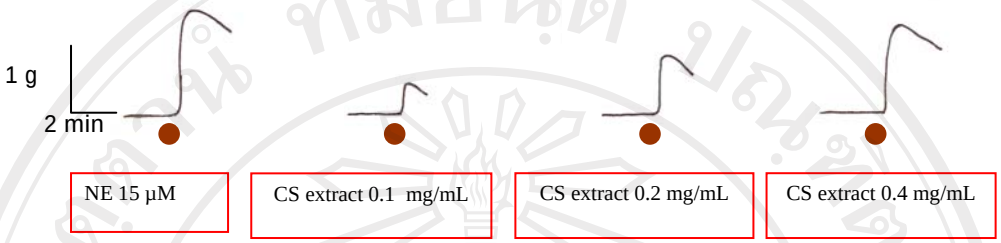

Figure 9. Effects of the CS extract and NE on human umbilical vein.

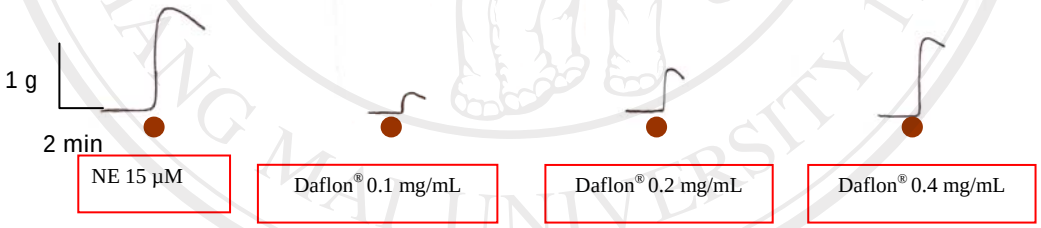

Figure 10. Effects of Daflon® and NE on human umbilical vein.

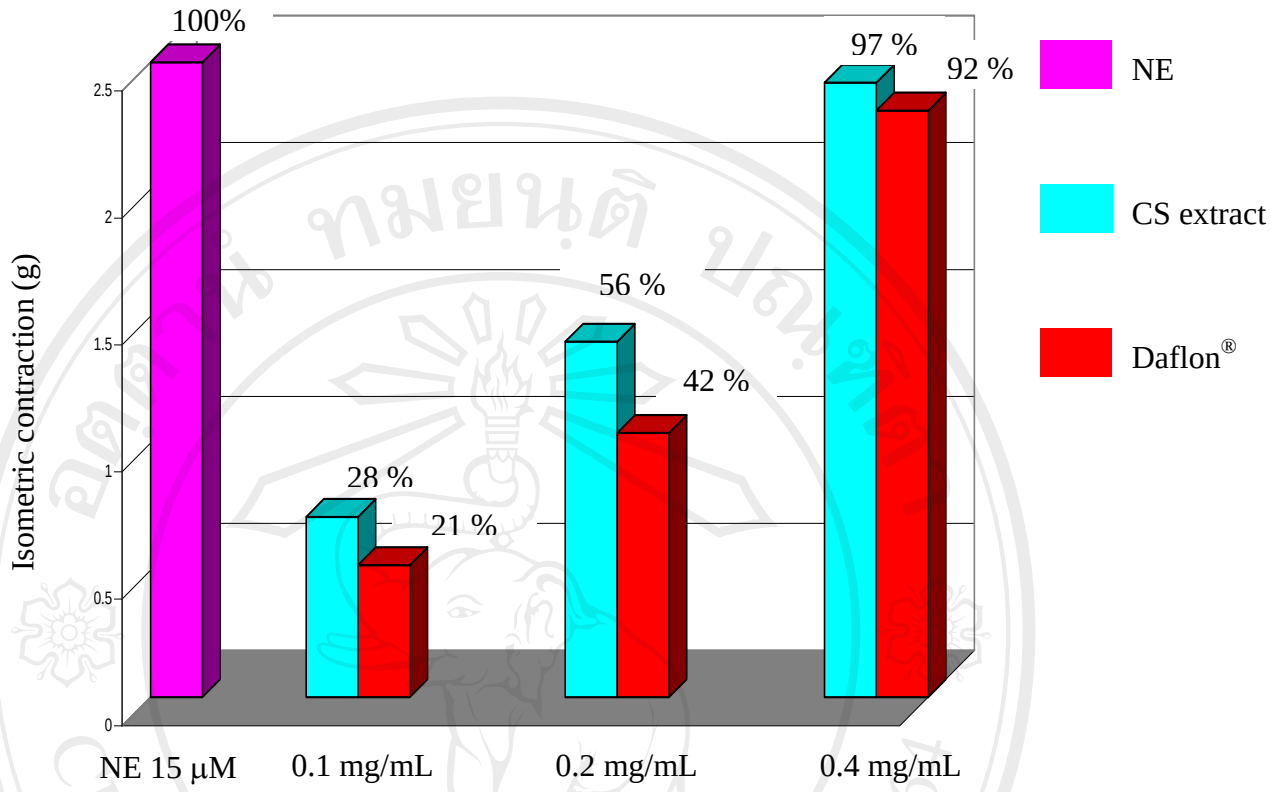

Figure 11. Vascular effect of the CS extract and Daflon® on isolated human umbilical vein as compared to NE. The results are presented as percent contraction of the CS extract and Daflon® as compared to that of NE (2.5g = 100%).