

Chapter III



Results

The actions of the crude extract from C. citratus on the isolated rat stomach and duodenum, and guinea-pig ileum segment were studied to confirm the action of crude extract. Increase contraction of stomach and small intestine and suitable for used as carminative and/or laxative drugs. The crude extract induces contraction of the isolated GI-tracts, as high as a contracture by acetylcholine, histamine or 5-HT. Contractile responses of each agonist and the crude extract were made for plotting the cumulative concentration-response curve. The results from the control and drugged conditions were then compared.

1. Effects of Crude extracts on Tone of Contraction of The Isolated Rat Stomach Fundus Strip.

Primary screening of the action of the crude extract was performed on the isolated rat stomach fundus strip only by recording tone of contraction. (The results obtained were shown in the Fig 5. A, B and Fig 6).

Fig 5 shows the contractile response of the isolated rat stomach fundus strip when exposed to different concentrations of the crude extract as 0.0625 - 1.00 g/ml; 0.3 ml (A) and concentrations

range 1:1 - 5:1 (g/ml); 0.3 ml (B) were measured in Tyrode's solution for 5 minutes after the addition of each concentration of the crude extract into the bath fluid. Administration of the crude extract in a cumulative regimen caused a concentration dependent which slight increase tone of contraction of the isolated rat stomach fundus strip. Additionally, Fig 6 shows the effect of the crude extract at concentrations range 1:1 - 5:1 is washed out concentration-response curve and was lesser than cumulative concentration-response curve significantly on tone of contraction of the isolated rat stomach fundus strip. The normal contraction could be recovered after washing the tissues with Tyrode's solution about five times.

The quantitative of cations and pH of each concentration of the crude extract were summarized in Table 2.

1.1 Effect of Crude extracts on Tone of Contraction of The Isolated Rat Stomach Fundus Strip Compared with The Other Spasmogens.

Spasmogens are drugs able to induce a contraction in muscle tissues, especially in smooth muscle such as acetylcholine, histamine, nicotine, barium chloride and 5-HT. All of which have a spasmogenic action on the isolated GI-tracts of rat and guinea-pig (Henderson et al., 1986). The present study found that the crude extract at concentrations of 0.25 g/ml and 1.00 g/ml; 0.2 and 0.3 ml caused variable increase in the contraction induced by acetylcholine, histamine and 5-HT. Application of acetylcholine (5.0×10^{-7} M. - 10.0

$\times 10^{-3}$ M.), histamine (5.0×10^{-6} M. - 10.0×10^{-2} M.) and 5-HT (5.0×10^{-9} M. - 10.0×10^{-5} M.) in a cumulative concentration regimen respectively into the bath fluid produced the contractile response of the isolated rat stomach fundus strip as shown in the control concentration-response curve of spasmogens. The addition of all concentrations of the crude extract caused significant concentration-dependent increase in the maximum effect of the control. The concentration-response curve of acetylcholine, histamine and 5-HT induce contraction of the isolated rat stomach fundus strip was shown in Fig 7, 8 and 9.

1.2 Effect of Crude extracts on Tone of Contraction of The Isolated Rat Stomach Fundus Strip Before and After Treated with Receptor Blocking Agents.

Receptor blocking agents using in this study were atropine, chlorpheniramine, cimetidine and cyproheptadine.

Application of the crude extract in a cumulative concentration regimen at concentrations range 1:1 - 5:1 (g:ml); 0.3 ml into the bath fluid produced a concentration-dependent contraction of the isolated rat stomach fundus strip. The crude extract induces contraction in this tissue which was not antagonized by atropine ($75 \mu\text{g/ml}$; 0.1 ml) significantly (Fig 10).

The contraction of the isolated rat stomach fundus strip induced by the crude extract at concentrations range 1:1 - 5:1; 0.3

ml, and was blocked by chlorpheniramine (75 µg/ml; 0.1 ml), cimetidine (75 µg/ml; 0.1 ml), chlorpheniramine plus cimetidine (75 µg/ml; 0.1 ml), 70% ethanol (0.1 ml) and 70% ethanol with cyproheptadine (17 µg/ml; 0.1 ml) significantly. The approximate decrease of the control percentage from the maximum contraction by 30%, 20%, 35% and 50%, and complete block were shown in Fig 11, 12, 13 and 14 respectively.

2. Effects of Crude extracts on Contraction of The Isolated Rat Duodenum Segment.

The crude extract from C. citratus were tested for their further effects on the isolated rat duodenum segment by measuring rate, amplitude and tone of spontaneous contraction. The results obtained were show in the Fig 15 and 16, and in the Table 3 and 4.

In the Table 3 demonstrates that all concentrations of the crude extract in a cumulative regimen into the bath fluid had not any effect on rate of contraction of the isolated rat duodenum segment significantly. The normal rate of contraction of the isolated rat duodenum segment was about 26-32 times per minute. Administration of the crude extract at same concentrations caused slight increase in the amplitude of contraction of the isolated rat duodenum segment (Table 4).

Spontaneous contraction of the isolated rat duodenum segment were measured in Tyrode's solution for 5 min after the addition of the crude extract at concentrations 0.0625 - 1.00 g/ml and concentrations range 1:1 - 5:1. Fig 15 shows that at concentrations

0.0625 - 1.00 g/ml; 0.3 ml of the crude extract (A.) caused very slow increase in tone of contraction of the isolated rat duodenum segment after administration of the crude extract in a cumulative regimen. Exception for the concentration 1.00 g/ml of the crude extract caused rapid increase in tone of spontaneous contraction. In Addition, at concentrations range 1:1 - 5:1; 0.3 ml of the crude extract (Fig 15 B.) caused slow increase of contraction. The normal contraction could be recovered after washing this tissues with Tyrode's solution about five times. The effect of the crude extract at concentrations range 1:1 - 5:1; 0.3 ml which washed out concentration-response curve was lesser than cumulative concentration-response curve significantly on tone of contraction (see Fig 16).

2.1 Effect of Crude extracts on Tone of Contraction of The Isolated Rat Duodenum Segment Compared with The Other Spasmogens.

Application of acetylcholine (5.0×10^{-8} M. - 10.0×10^{-5} M.) histamine (5.0×10^{-8} M. - 10.0×10^{-4} M.) or 5-HT (5.0×10^{-9} M. - 10.0×10^{-5} M.) into the bath fluid produced the contractile response of the isolated rat duodenum segment (The control concentration-response curve of spasmogens). The addition of all concentrations (0.25 and 1.00 g/ml; 0.2 and 0.3 ml) of the crude extract caused significantly concentration-dependent increasing of the maximum effect of the control concentration-response curve of acetylcholine, histamine or 5-HT which induced contraction of the isolated rat duodenum segment. The results obtained were shown in the Fig 17, 18 and 19.

2.2 Effect of Crude extracts on Tone of Contraction of The Isolated Rat Duodenum Segment Before and After Treated with Receptor Blocking Agents.

The contraction of the isolated rat duodenum segment induced by the crude extract at concentrations range 1:1 - 5:1 (g:ml); 0.3 ml in a cumulative regimen into the bath fluid could not be blocked by cimetidine (75 $\mu\text{g/ml}$) significantly (Fig 20). This effect has been blocked by atropine, chlorpheniramine and chlorpheniramine plus cimetidine, all at the same concentration of 75 $\mu\text{g/ml}$, 70% ethanol or 70% ethanol with cyproheptadine (17 $\mu\text{g/ml}$) significantly. The approximate decrease in the control percentage maximum contraction were 60%, 40%, 50%, 30%, and 60% respectively (Fig 21, 22, 23, and 24). This results indicate that the contraction of the isolated rat duodenum segment induced by the crude extract was not mediated via H_2 - receptor but mediated via ACh-, H_1 - and 5-HT receptors respectively.

3. Effects of Crude extracts on Tone of Contraction of The Isolated Guinea-pig Ileum Segment.

The action of the crude extract was tested on the isolated guinea-pig ileum segment only by measuring tone of contraction as same as the isolated rat stomach fundus strip. Fig 25 A and B demonstrates that administration of the crude extract at concentrations range 1:1 - 5:1 (g:ml); 0.3 ml in a cumulative regimen into the bath fluid caused increase in tone of contraction of the isolated guinea-pig ileum segment (A), and this effect was blocked by atropine (75 $\mu\text{g/ml}$) (B).

Fig 26 shows the effect of the crude extract at concentrations range 1:1 - 5:1 (0.3 ml). The concentration-response curve was lesser than cumulative concentration-response curve significantly on tone of contraction of the isolated guinea-pig ileum segment.

3.1 Effect of Crude extracts and Coaxial Electrical Stimulation on Amplitude of Contraction of The Isolated Guinea-pig Ileum Segment.

Studying effect of the crude extract and coaxial electrical stimulation on amplitude of contraction of the isolated guinea-pig ileum segment were shown in the Fig 27, 28 and 29.

The crude extract at concentrations 3:1 and 5:1 (g : ml); 0.3 ml caused significantly increase in amplitude of contraction of the isolated guinea-pig ileum segment when compared with control amplitude of contraction of coaxial electrical stimulating at 3, 5 and 10 volts respectively. The control amplitude of contraction of isolated guinea-pig ileum segment with electrical stimulation at 3, 5 and 10 volts were 7.6 ± 2.36 , 10.2 ± 2.39 and 19.2 ± 2.74 mm respectively. After treated with the crude extract at concentration 3:1 in Tyrode's solution for 10 minutes, the increase in amplitude of contraction with electrical stimulated at 3, 5 and 10 volts were 17.3 ± 2.05 , 16.5 ± 2.01 and 24.8 ± 3.04 mm respectively (Fig 27). Additionally, the crude extract at concentration 5:1 caused significant increase in control amplitude of contraction. For electrical stimulation at 3, 5 and 10 volts were 7.9 ± 2.51 (19.0 ± 2.49), 10.3 ± 2.49 (17.7 ± 2.62)

and 19.0 ± 2.00 (25.8 ± 3.96) mm respectively (see Fig 28).

Fig 29 shows bar graphs of amplitude of contraction of isolated guinea-pig ileum segment when stimulated at 3, 5 and 10 volts, before and after application with the crude extract at concentrations 3:1 and 5:1. In this figure indicates that the crude extracts caused significant increase in amplitude of contraction when compared with the control amplitude which induced by coaxial electrical stimulation.

3.2 Effect of Crude extracts on Tone of Contraction of The Isolated Guinea-pig Ileum Segment Before and After Treated with Receptor Blocking Agents.

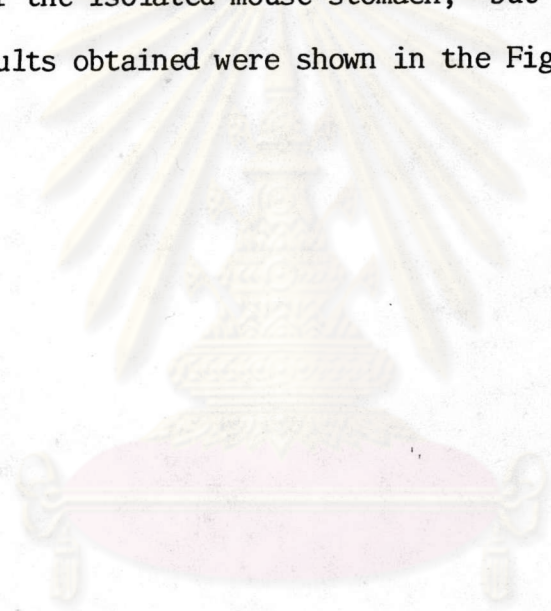
Application of the crude extract into a cumulative concentration regimen at concentrations range 1:1 - 5:1; 0.3 ml to the bath fluid produced the contractile response of the isolated guinea-pig ileum segment, and this effect could not be blocked by cimetidine (75 $\mu\text{g}/\text{ml}$) significantly (Fig 32).

The contraction of the isolated guinea-pig ileum segment induced by the crude extract at concentrations range 1:1 - 5:1; 0.3 ml, have been blocked by atropine (75 $\mu\text{g}/\text{ml}$), chlorpheniramine (75 $\mu\text{g}/\text{ml}$), chlorpheniramine plus cimetidine (75 $\mu\text{g}/\text{ml}$), 70% ethanol or 70% ethanol with cyproheptadine (17 $\mu\text{g}/\text{ml}$) significantly. The approximate decrease of the control percentage maximum contraction were 90%, 15%, 10%, 50% and 80% respectively. (show in Fig 30, 31, 33 and

34.)

4. Effect of Crude extracts on Tone of Contraction on Mucosal and Serosal side of The Isolated Mouse Stomach.

The addition of the crude extract in a cumulative concentration regimen at concentrations range 1:1 -5:1; 0.3 ml into the bath fluid at mucosal side caused significant increase in tone of contraction of the isolated mouse stomach, but no effect on serosal side. The results obtained were shown in the Fig 35 and 36.



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Table 2 The quantitative of cations (Na^+ , K^+ and Ca^{2+} ; in $\mu\text{g/ml}$) and pH of each concentration of the crude extracts from C. citratus.

Concentrations of the crude extract from <u>C. citratus</u>		Quantitative of cation ($\mu\text{g/ml}$)			pH
		Na^+	K^+	Ca^{2+}	
0.0625	g/ml	0.0046	0.14	21.76	6.14
0.125	g/ml	0.0076	0.21	32.38	6.13
0.250	g/ml	0.0080	0.32	64.72	6.05
0.500	g/ml	0.0130	0.46	108.23	5.95
1.00	g/ml (1:1)	0.0230	0.64	127.83	5.88
2.00	g/ml (2:1)	0.0270	1.32	142.15	5.69
3.00	g/ml (3:1)	0.0360	1.49	161.66	5.67
4.00	g/ml (4:1)	0.0500	2.51	183.94	5.55
5.00	g/ml (5:1)	0.0700	3.62	207.71	5.43

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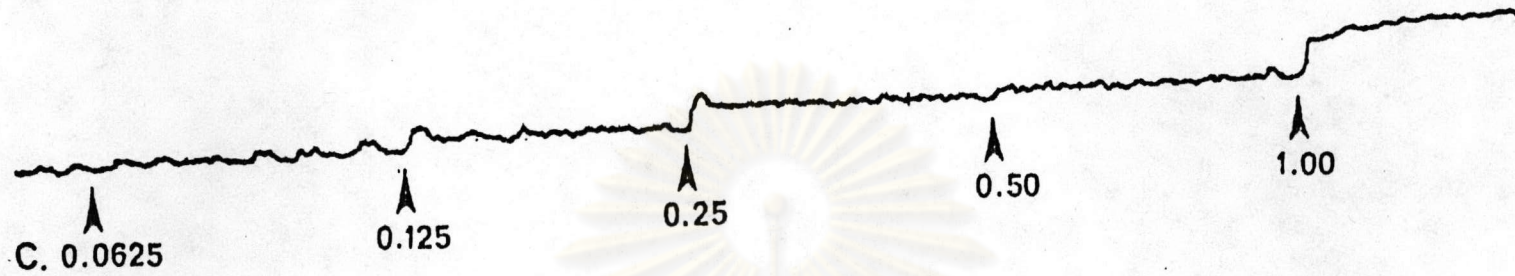
Table 3. Effect of crude extracts from C. citratus on rate of contraction of the isolated rat duodenum segment. (n=10) All concentrations of the crude extract did not affect on rate of contraction of the isolated rat duodenum segment significantly.

Concentrations of the crude extract from <u>C. citratus</u> .		Rate of contraction (time/minute)										mean+S.D.	
0.2	ml	Control	30	31	26	29	30	31	31	28	30	31	29.7+0.01
0.0625	g/ml		30	31	26	29	30	31	31	28	30	31	29.7+0.01
0.125	g/ml		31	30	26	29	30	31	31	27	30	31	29.6+0.01
0.25	g/ml		30	30	26	29	30	31	31	28	31	31	29.7+0.01
0.50	g/ml		30	30	26	30	30	31	31	28	30	31	29.7+0.01
1.00	g/ml		31	31	26	29	30	31	31	27	30	31	29.7+0.01
0.3	ml	Control	26	27	30	29	31	29	28	31	30	30	29.1+0.01
0.0625	g/ml		26	27	30	29	31	29	28	31	30	30	29.1+0.01
0.125	g/ml		26	28	30	28	31	29	28	31	31	30	29.2+0.01
0.25	g/ml		26	28	31	28	31	29	28	31	30	30	29.2+0.01
0.50	g/ml		26	27	31	29	30	29	28	30	30	30	29.0+0.01
1.00	g/ml		26	27	31	30	31	29	28	31	30	30	29.2+0.01
0.3	ml	Control	32	31	31	30	29	32	28	31	31	28	30.3+1.49
1:1	(g:ml)		32	31	31	30	29	32	28	31	31	28	30.3+1.49
2:1			32	32	32	30	29	32	28	31	31	29	30.6+1.50
3:1			32	31	32	31	29	31	28	31	31	28	30.4+1.50
4:1			32	31	31	30	29	32	29	31	31	28	30.4+1.34
5:1			32	31	31	30	29	32	29	31	31	29	30.5+1.17

Table 4. Effect of crude extracts from C. citratus on amplitude of contraction of the isolated rat duodenum segment. (n=10) All concentrations of the crude extract did not affect on amplitude of contraction of the isolated rat duodenum segment significantly.

Concentrations of the crude extract from <u>C. citratus</u> .		Amplitude of contraction (g)										mean+S.D.	
0.2 ml	Control	0.85	0.90	0.80	1.00	0.75	0.86	0.93	1.00	0.85	0.75	0.869	+0.89
0.0625 g/ml		1.00	1.20	0.80	1.15	0.90	0.95	1.00	1.20	1.00	0.95	1.015	+1.31
0.125 g/ml		1.00	1.20	0.80	1.20	0.90	1.00	1.15	1.20	1.15	1.00	1.060	+1.41
0.25 g/ml		1.15	1.35	0.95	1.30	0.80	1.00	1.20	1.35	1.15	1.00	1.125	+1.84
0.50 g/ml		1.20	1.35	1.10	1.40	0.85	1.00	1.20	1.40	1.20	1.15	1.185	+1.74
1.00 g/ml		3.15	1.40	1.65	1.85	0.85	1.65	1.20	2.15	1.43	1.30	1.663	+6.33
0.3 ml	Control	1.05	1.20	1.00	0.80	0.95	1.00	1.30	0.90	1.00	0.85	1.005	+1.51
0.0625 g/ml		1.05	1.20	1.00	0.80	0.95	1.00	1.30	0.90	1.00	0.85	1.005	+1.51
0.125 g/ml		.975	1.20	1.05	0.80	1.10	1.30	1.30	0.95	1.05	0.85	1.057	+1.72
0.25 g/ml		.975	1.20	1.05	0.85	1.10	1.35	1.30	1.00	1.05	0.90	1.077	+1.63
0.50 g/ml		1.00	1.20	1.10	0.90	1.25	1.30	1.45	1.10	1.10	0.90	1.130	+1.75
1.00 g/ml		1.05	1.20	1.65	1.10	1.60	1.30	1.60	1.45	1.20	1.35	1.350	+2.17
0.3 ml	Control	1.50	0.45	0.40	0.90	0.85	0.45	0.60	0.55	0.40	0.50	0.660	+3.43
1:1 (g:ml)		0.90	1.00	1.80	1.15	0.85	1.60	0.55	1.20	1.25	0.95	1.125	+3.66
2:1		1.10	1.70	2.20	1.15	0.90	2.15	0.70	1.85	1.25	1.25	1.425	+5.19
3:1		0.95	2.05	2.00	1.30	1.15	2.30	0.95	2.10	1.40	1.30	1.550	+5.10
4:1		1.15	2.30	1.50	1.40	1.05	2.35	1.05	2.10	1.35	1.30	1.555	+5.04
5:1		0.95	1.80	1.30	1.45	1.05	2.10	1.10	2.05	1.35	1.35	1.455	+4.13

A.



B.

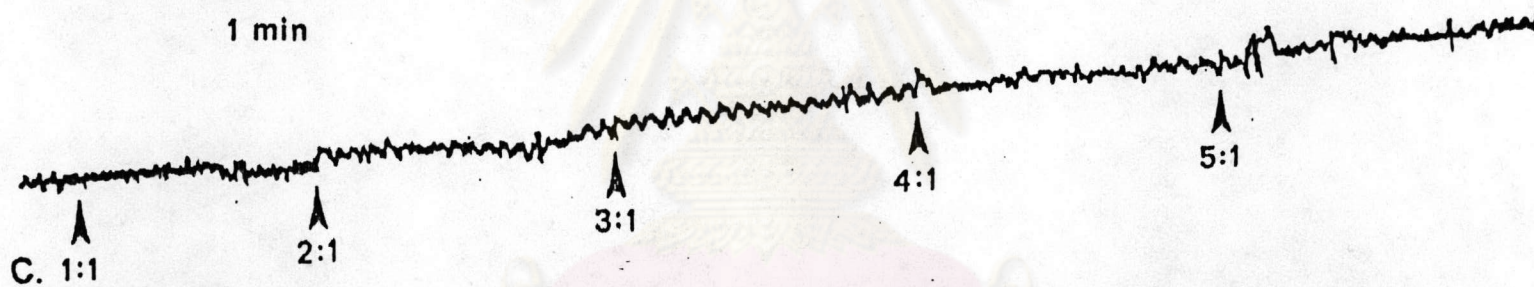


Fig 5

Trace of cumulative dose-response relationship of the crude extract from *C. Citratus* at concentrations 0.0625-1.00 g/ml; 0.3 ml (A) and concentrations range 1:1-5:1 (g:ml); 0.3 ml (B) on the isolated rat stomach fundus strip. The record shows the spasmogenic effect of the crude extracts on tone of contraction. The calibration of 1 g tension is show on the vertical line and time scale under the first row of recordings and is identical for all cases.

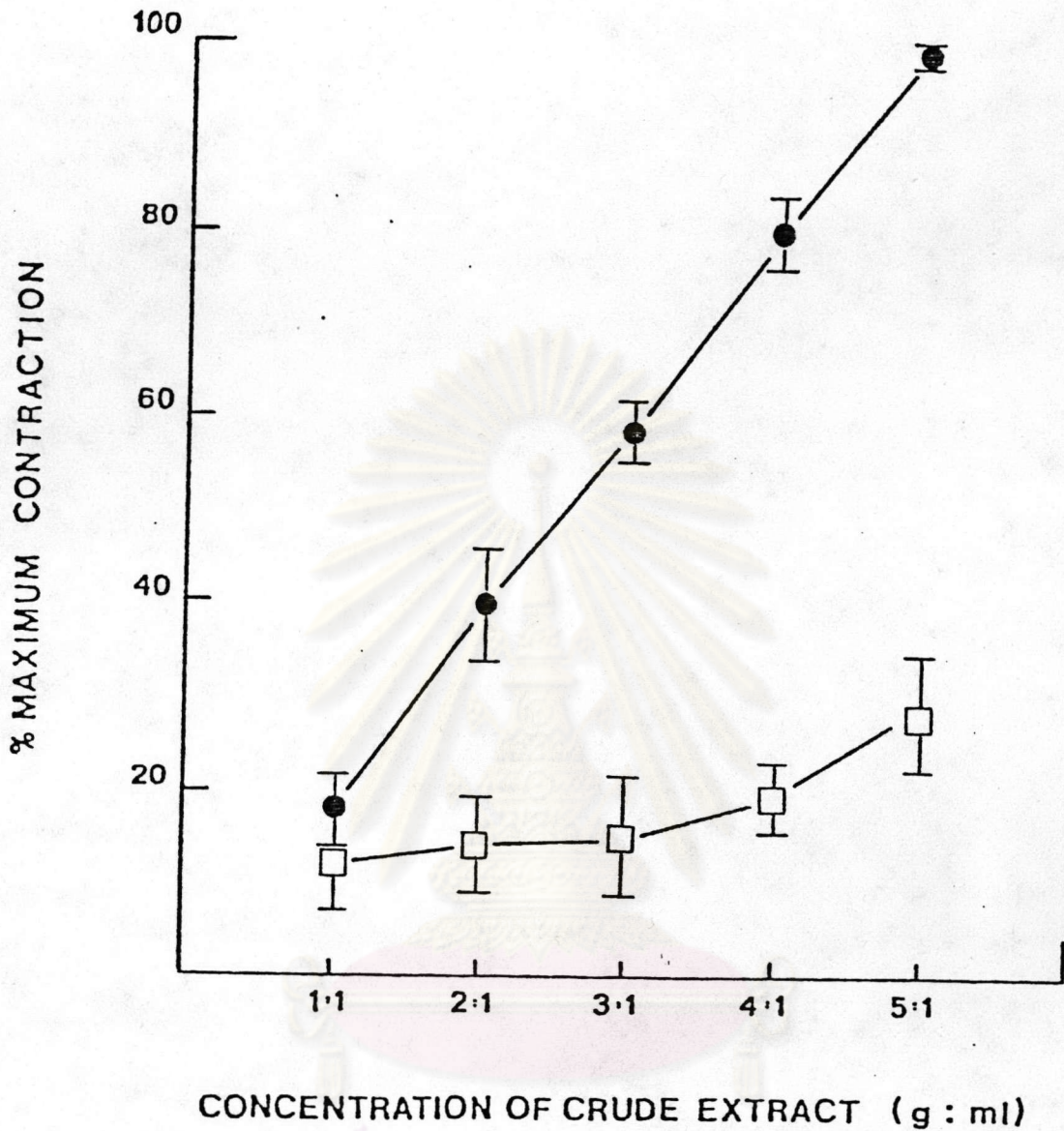


Fig 6 The effect of the crude extract from C. citratus at concentrations range 1:1 - 5:1 (g:ml) on tone of contraction of the isolated rat stomach fundus strip in a cumulative dose regimen (●) and in washed out each dose every 5 min of treatment (□). The effect of all concentrations of the crude extract on washed out concentration-response curve is lesser than cumulative concentration-response curve significantly on tone of contraction of the isolated rat stomach fundus strip . ($p < 0.001$)



Fig 7 Cumulative concentration-response curves of acetylcholine on tone of contraction of the isolated rat stomach fundus strip following preincubation for 10 min with the crude extract from C. citratus at concentration 0.25 g/ml; 0.2 ml (□), 0.25 g/ml; 0.3 ml (●), 1.00 g/ml; 0.2 ml (☆) and 1.00 g/ml; 0.3 ml (★). Acetylcholine induced sustained contraction of the isolated rat stomach fundus strip. Each point is the mean response of at least 8 measurements; vertical line show standard deviation of the mean (S.D.). The ordinate scale shows response as percentage of maximum contraction and the abscissa scale is the concentrations range 5.0×10^{-7} M - 10.0×10^{-3} M of acetylcholine. All points on the curves from all concentrations of the crude extract treated condition are significantly different from the control curve. (★—★). (P<0.001, Student's t-test)

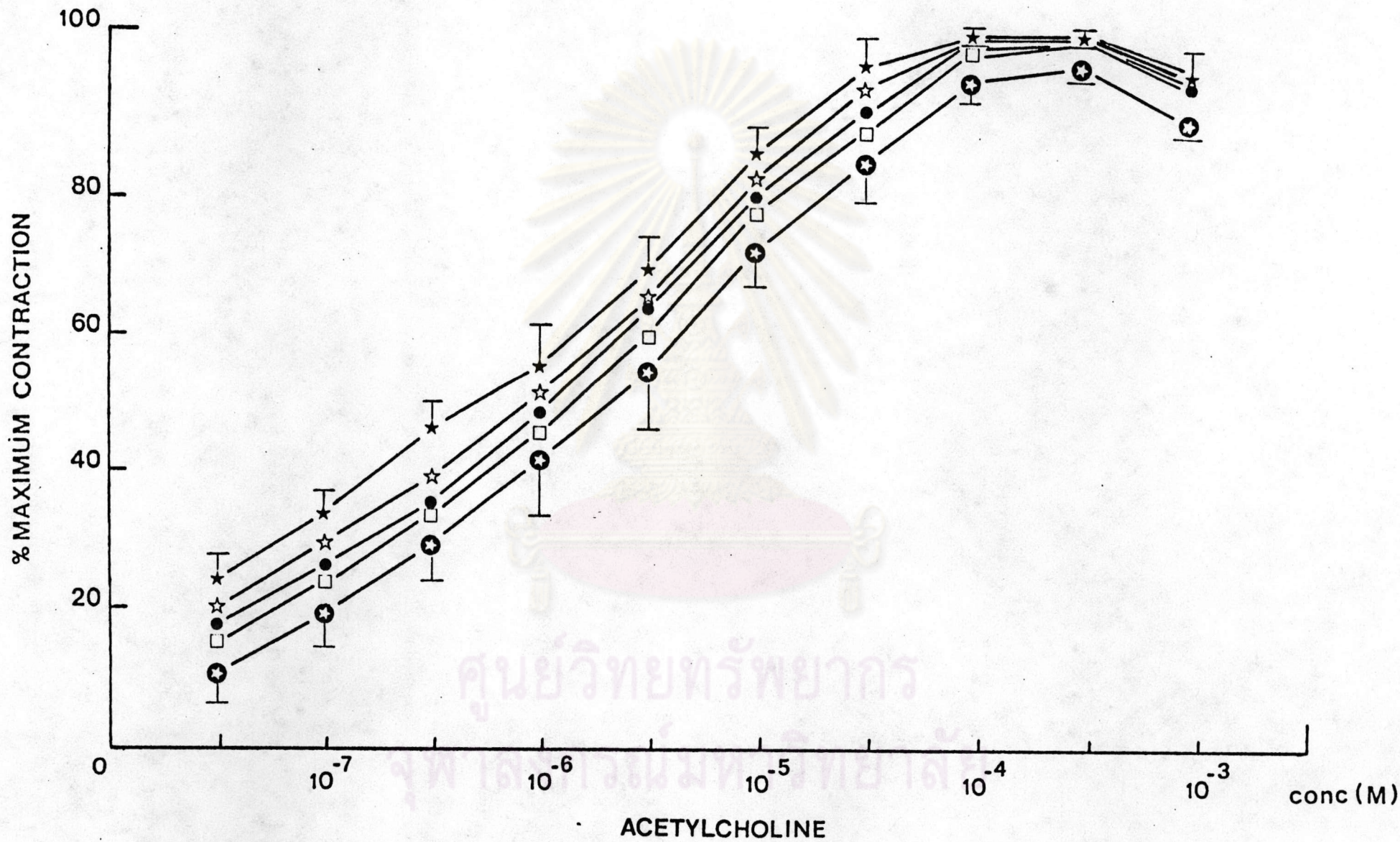


Fig 7

Fig 8 Cumulative concentration-response curves of histamine on tone of contraction of the isolated rat stomach fundus strip following preincubation for 10 min with the crude extract from C. citratus at concentration 0.25 g/ml; 0.2 ml (□), 0.25 g/ml; 0.3 ml (●), 1.00 g/ml; 0.2 ml (☆) and 1.00 g/ml; 0.3 ml (★). Histamine induced sustained contraction of the isolated rat stomach fundus strip. Each point is the mean response of at least 8 measurements; vertical line show standard deviation of the mean (S.D.). The ordinate scale shows response as percentage of maximum contraction and the abscissa scale is the concentrations range 5.0×10^{-6} M - 10.0×10^{-2} M of histamine. All points on the curves from all concentrations of the crude extract treated condition are significantly different from the control curve.

(★ ———— ★). ($P < 0.001$, Student's t-test)

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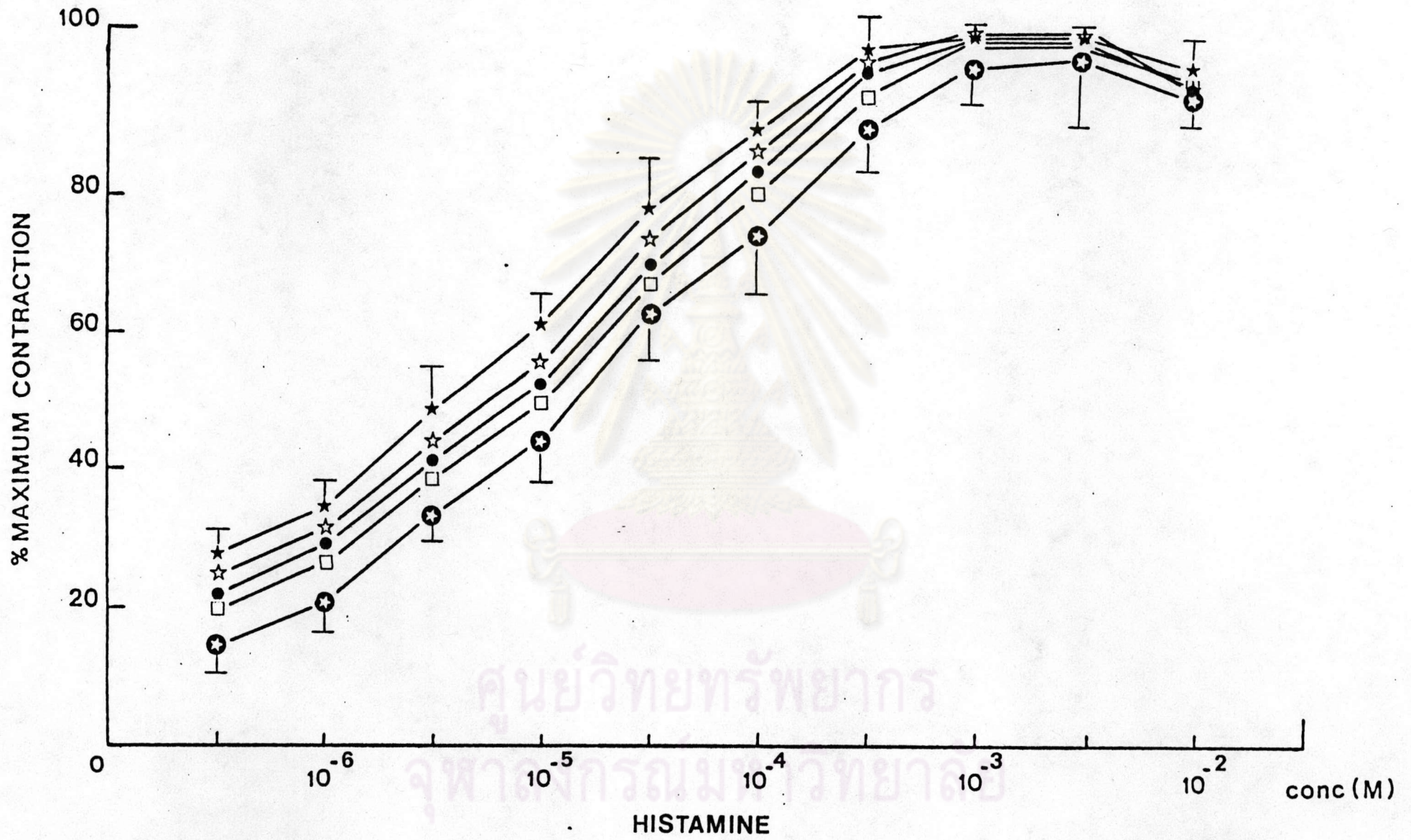


Fig 8

Fig 9 Cumulative concentration-response curves of 5-HT on tone of contraction of the isolated rat stomach fundus strip following preincubation for 10 min with the crude extract from C. citratus at concentration 0.25 g/ml; 0.2 ml (□), 0.25 g/ml; 0.3 ml (●), 1.00 g/ml; 0.2 ml (☆) and 1.00 g/ml; 0.3 ml (★). 5-HT induced sustained contraction of the isolated rat stomach fundus strip. Each point is the mean response of at least 8 measurements; vertical line show standard deviation of the mean (S.D.). The ordinate scale shows response as percentage of maximum contraction and the abscissa scale is the concentrations range 5.0×10^{-9} M - 10.0×10^{-5} M of 5-HT. All points on the curves from all concentrations of the crude extract treated condition are significantly different from the control curve. (★—★). (P<0.001, Student's t-test)

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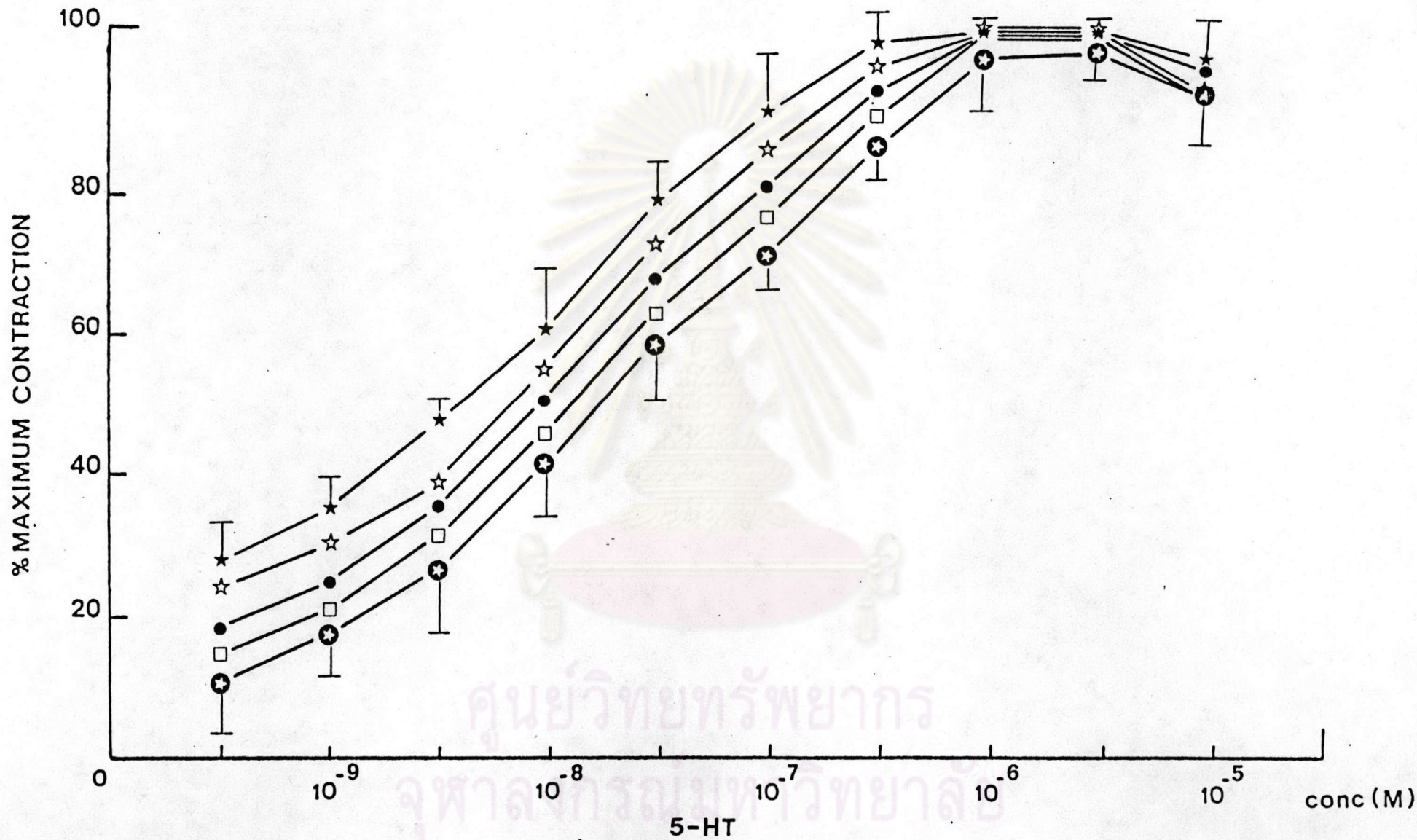


Fig 9

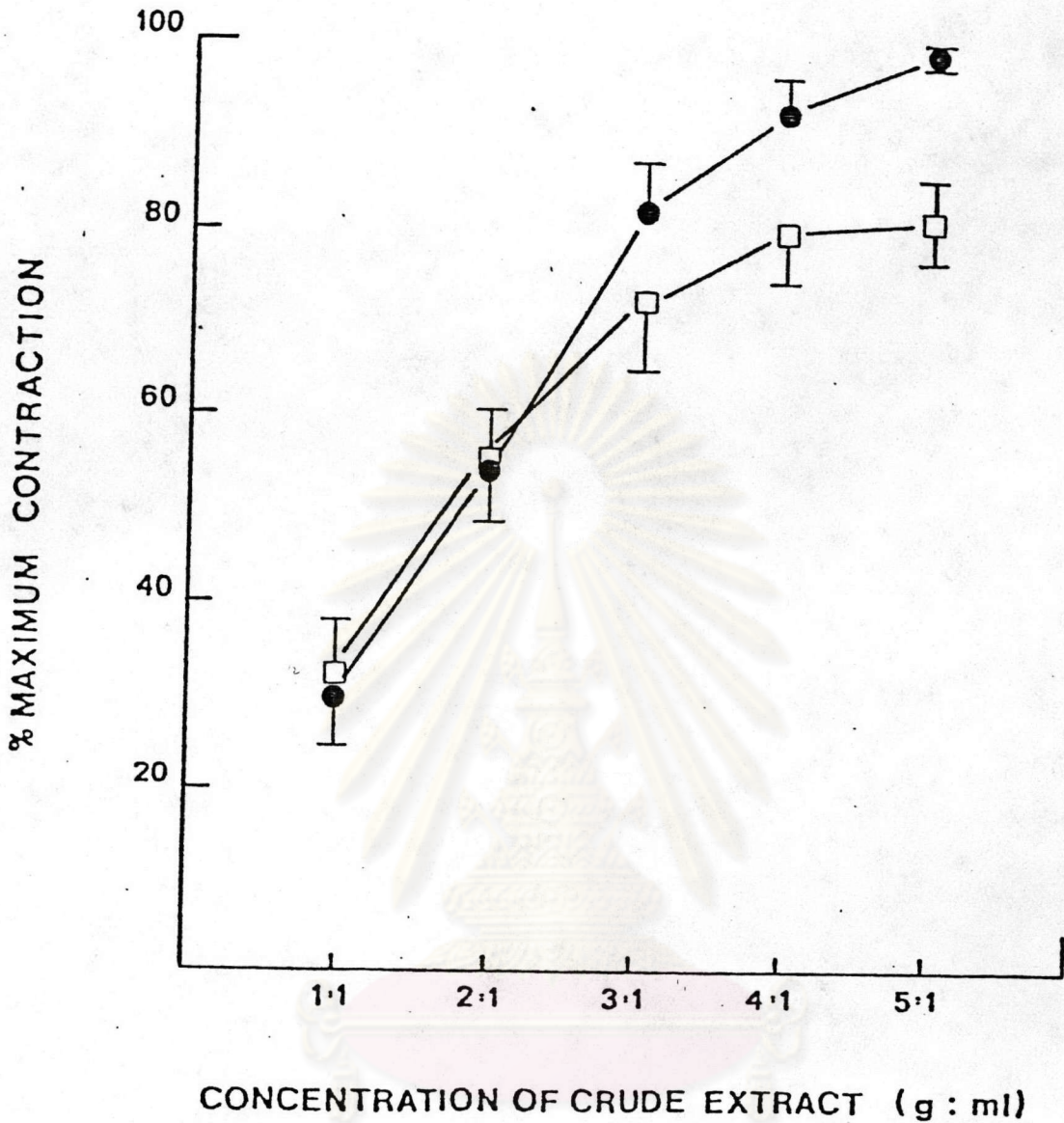


Fig 10 Cumulative concentration-response curves of the crude extract from *C. citratus* at concentrations range 1:1 - 5:1 (g:ml) on tone of contraction of the isolated rat stomach fundus strip in the absence (●) and presence of atropine at concentration 75 µg/ml (□), which preincubated for 10 min. All concentrations of the crude extract induced sustained contraction of the isolated this tissue, incubation of the tissue with atropine produced non-significantly block of the contractile response of the crude extracts. (n=8)

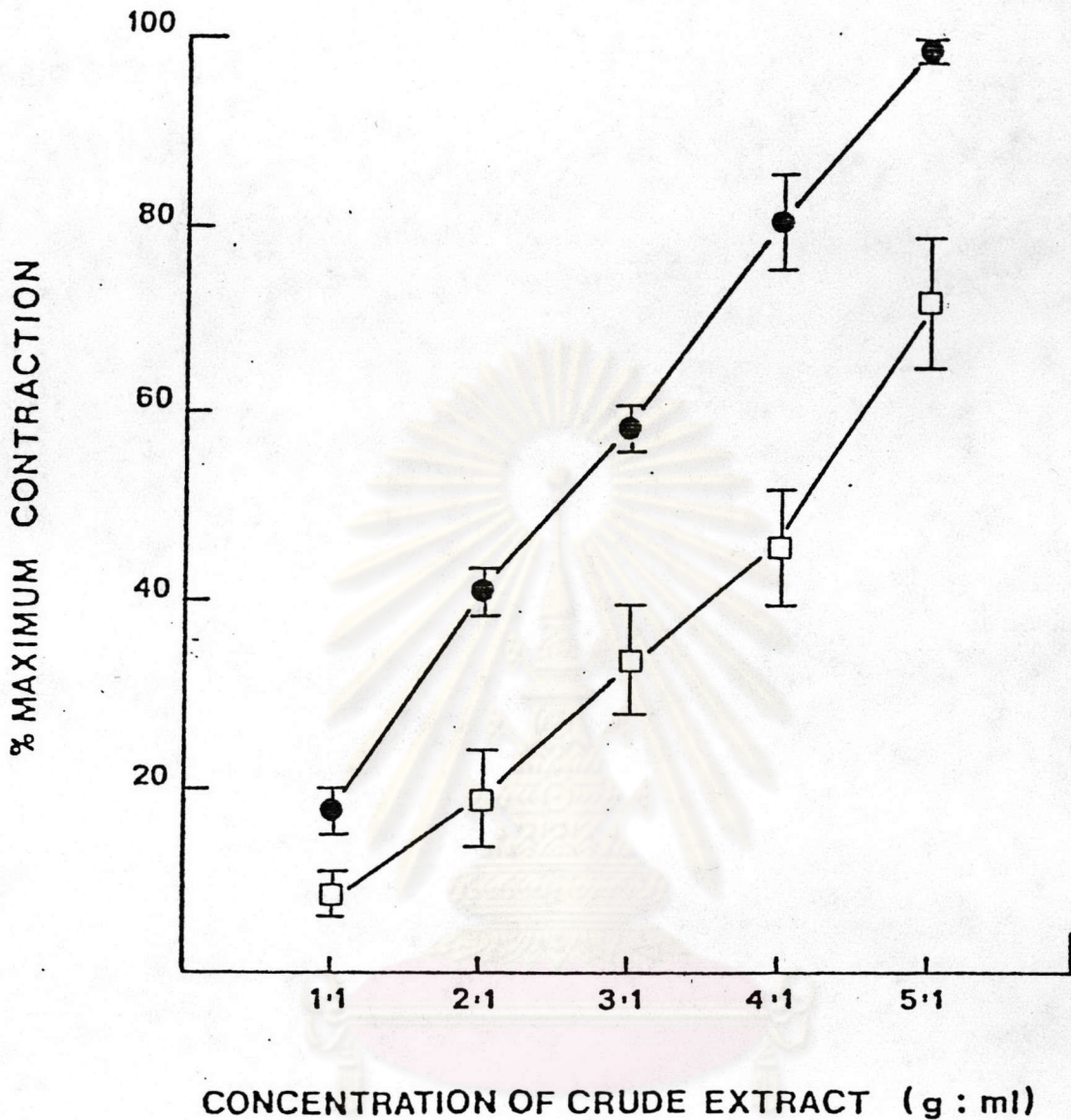


Fig 11 Cumulative concentration-response curves of the crude extract from *C. citratus* at concentrations range 1:1 - 5:1 (g:ml) on tone of contraction of the isolated rat stomach fundus strip in the absence (●) and presence of chlorpheniramine at concentration 75 µg/ml (□), which preincubated for 10 min. All concentrations of the crude extract induced sustained contraction of the isolated this tissue, incubation of the tissue with chlorpheniramine produced significantly block of the contractile response of the crude extracts. ($p < 0.001$) (n=8) .

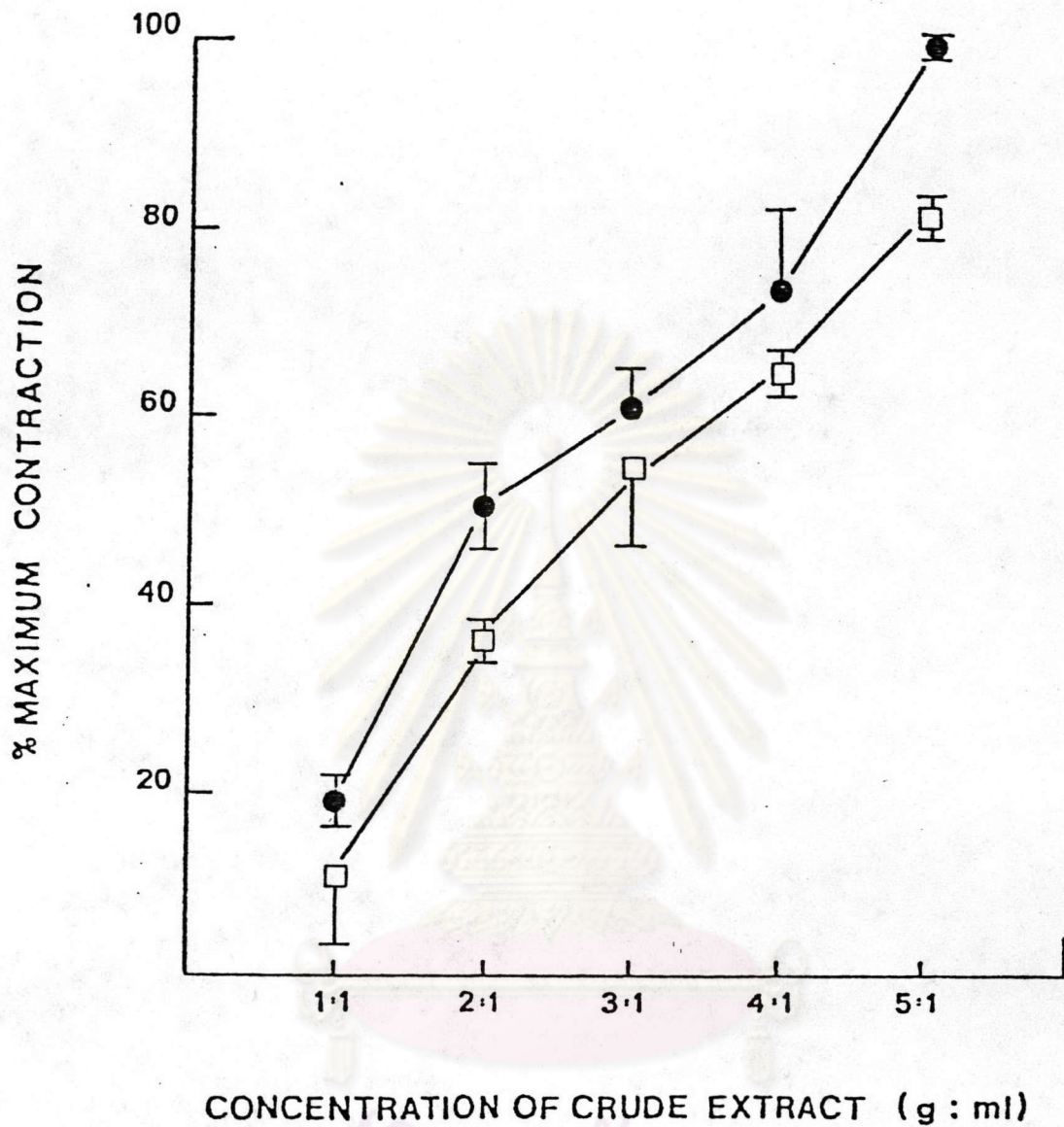


Fig 12 Cumulative concentration-response curves of the crude extract from *C. citratus* at concentrations range 1:1 - 5:1 (g:ml) on tone of contraction of the isolated rat stomach fundus strip in the absence (●) and presence of cimetidine at concentration 75 µg/ml (□), which preincubated for 10 min. All concentrations of the crude extract induced sustained contraction of the isolated this tissue, incubation of the tissue with cimetidine produced significantly block of the contractile response of the crude extracts. ($p < 0.005$) ($n=8$)

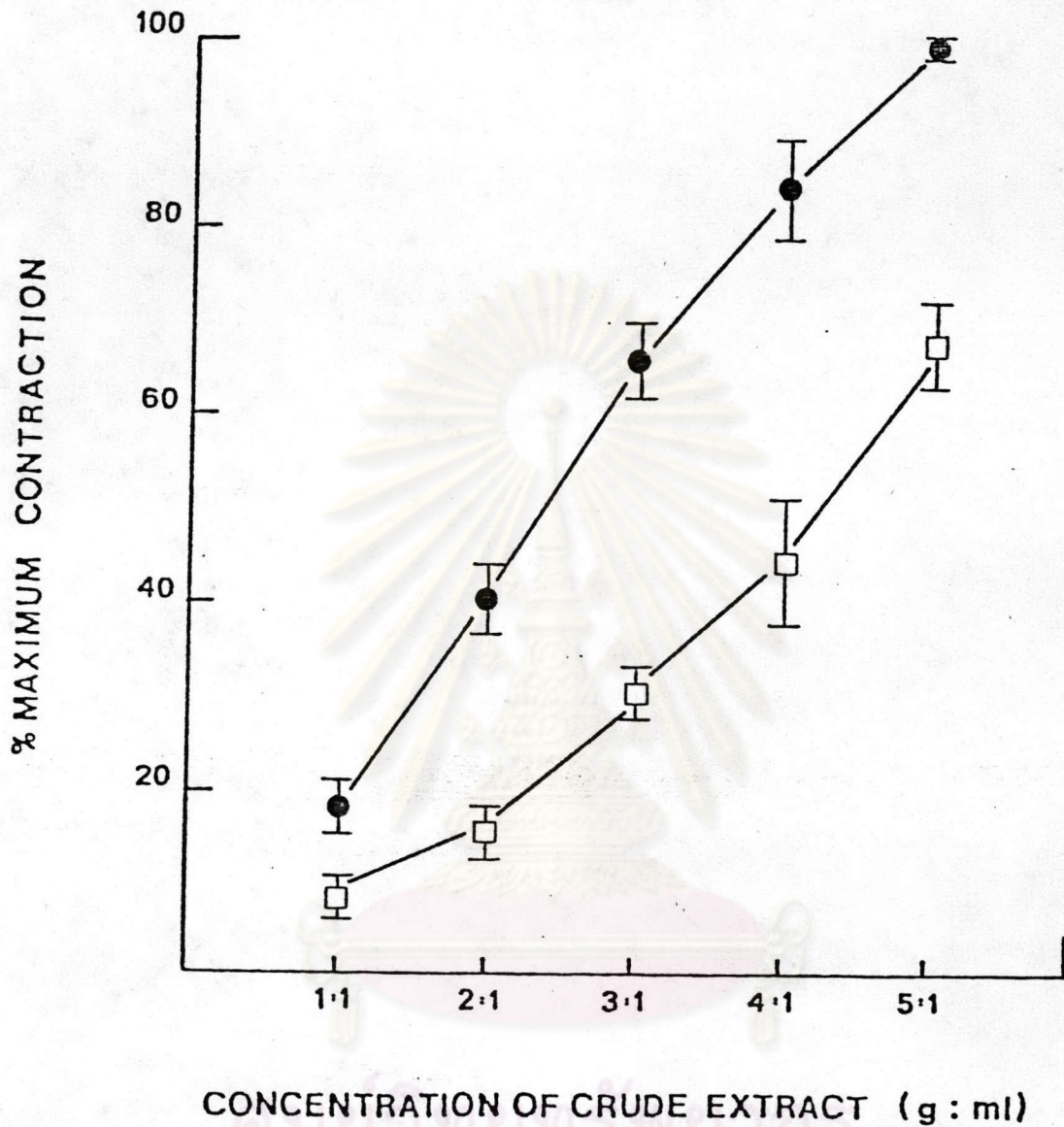


Fig 13 Cumulative concentration-response curves of the crude extract from *C. citratus* at concentrations range 1:1 - 5:1 (g:ml) on tone of contraction of the isolated rat stomach fundus strip in the absence (●) and presence of chlorpheniramine and cimetidine at concentration 75 μ g/ml (□), which preincubated for 10 min. All concentrations of the crude extract induced sustained contraction of the isolated this tissue, incubation of the tissue with chlorpheniramine and cimetidine produced significantly block of the contractile response of the crude extracts. ($p < 0.005$) ($n=8$)

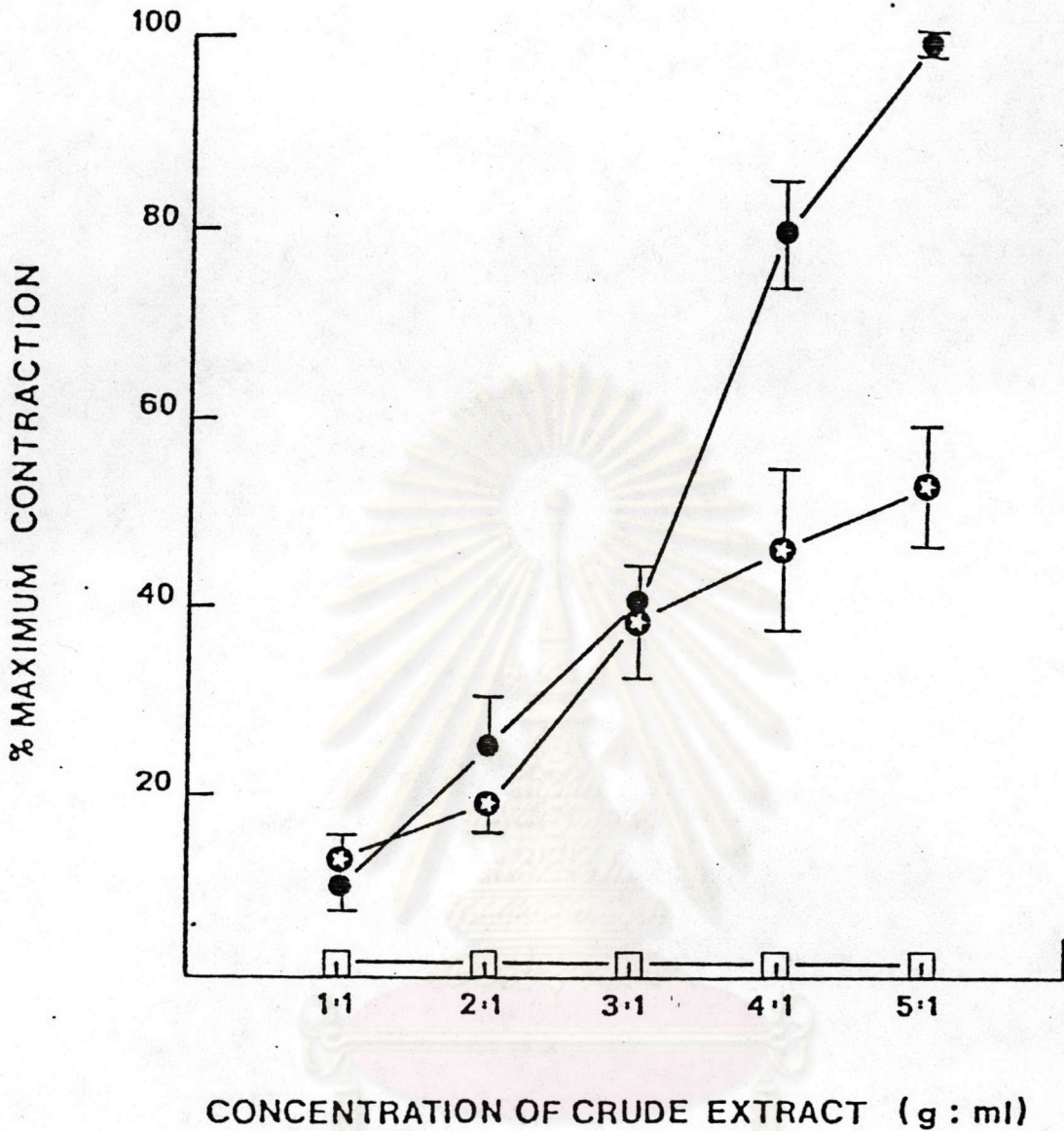


Fig 14 Cumulative concentration-response curves of the crude extract from *C. citratus* at concentrations range 1:1 - 5:1 (g:ml) on tone of contraction of the isolated rat stomach fundus strip in the absence (●) and presence of cyproheptadine (75 µg/ml) with 70% ethanol (□), and 70% ethanol (⊕), which preincubated for 10 min. All concentrations of the crude extract induced sustained contraction of the isolated this tissue, incubation of the tissue with cyproheptadine and 70% ethanol produced complete significantly block of the contractile response of the crude extracts (NS = non-significant). (n=10)

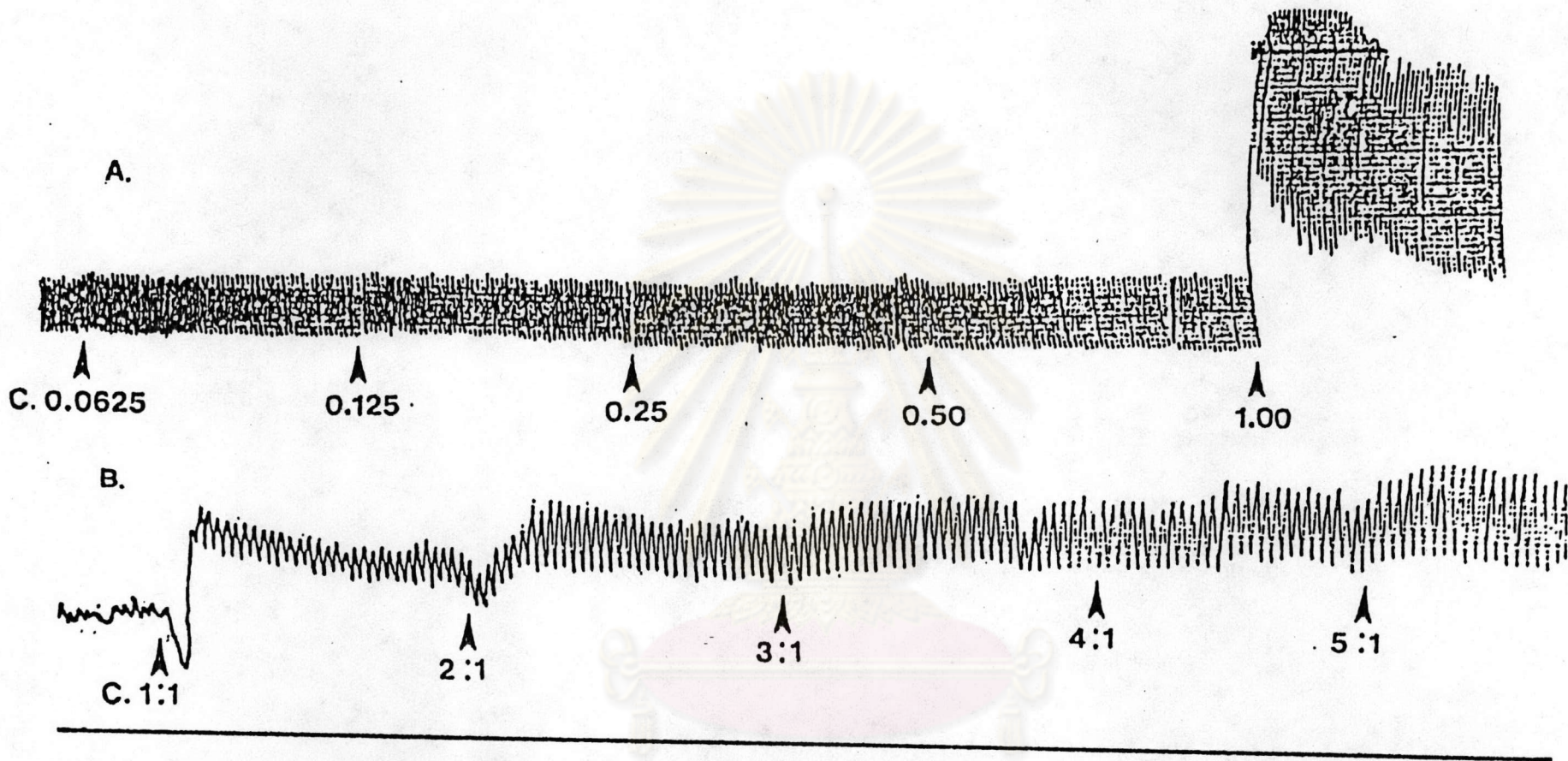


Fig 15 Trace of cumulative dose-response relationship of the crude extract from *C. citratus* at concentrations 0.0625 - 1.00 g/ml; 0.3 ml (A.) and concentrations range 1:1 - 5:1 (g:ml); 0.3 ml (B.) on the isolated rat duodenum segment. The record shows the spasmogenic effect of the crude extracts on tone of contraction. All concentrations of crude extract caused slight increase tone of contraction, except at concentration 1.00 g/ml (A.) caused rapid increase tone of contraction of the isolated rat duodenum segment.

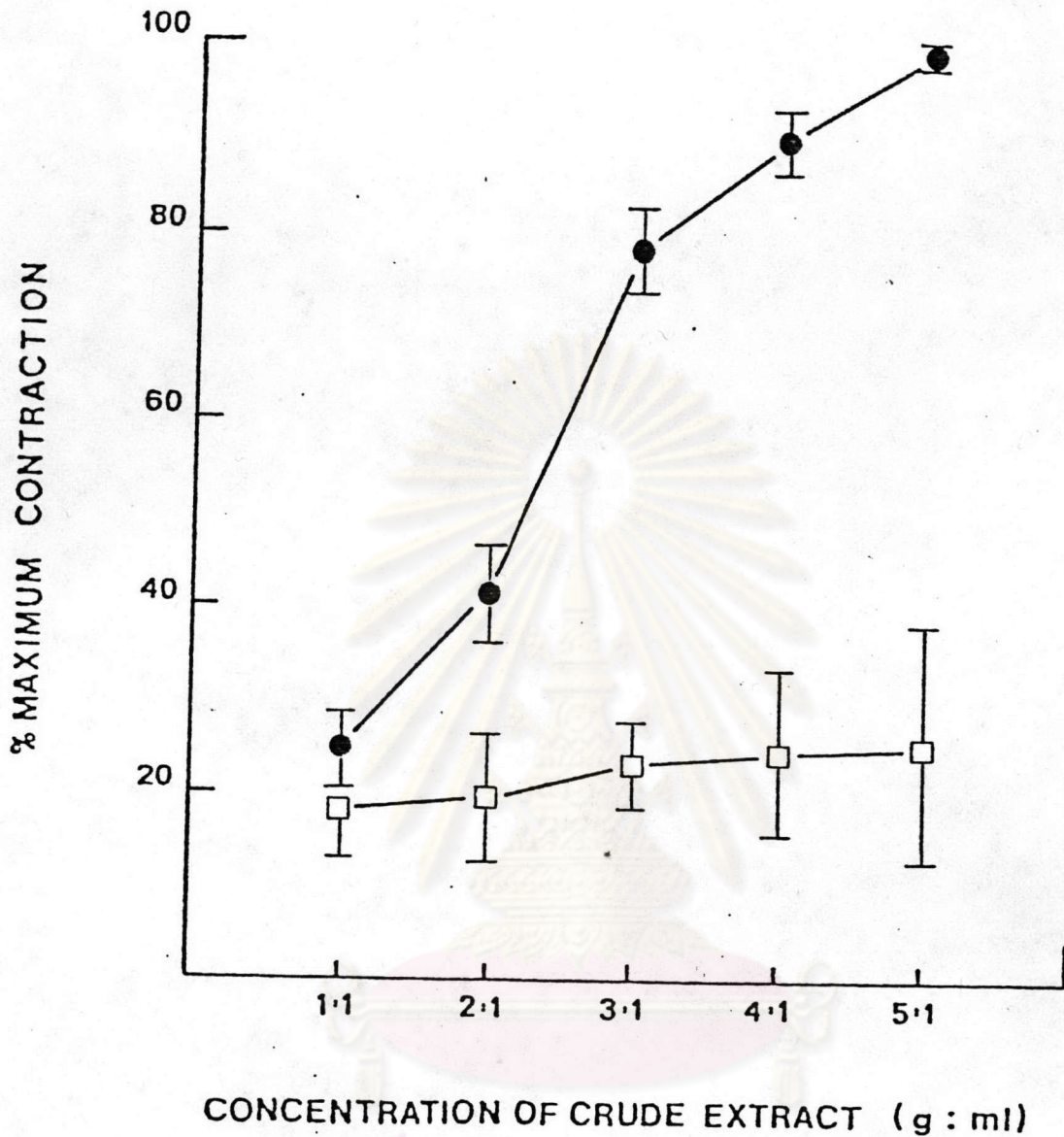


Fig 16 The effect of the crude extract from *C. citratus* at concentrations range 1:1 - 5:1 (g:ml) on tone of contraction of the isolated rat duodenum segment in a cumulative Jose regimen (●) and in washed out each dose every 5 min of treatment (□). The effect of all concentrations of the crude extract on washed out concentration-response curve is lesser than cumulative concentration-response curve significantly on tone of contraction of the isolated rat duodenum segment. ($p < 0.001$)

Fig 17 Cumulative concentration-response curves of acetylcholine on tone of contraction of the isolated rat duodenum segment following preincubation for 10 min with the crude extract from C. citratus at concentration 0.25 g/ml; 0.2 ml (□), 0.25 g/ml; 0.3 ml (●), 1.00 g/ml; 0.2 ml (☆) and 1.00 g/ml; 0.3 ml (★). Acetylcholine induced sustained contraction of the isolated rat stomach fundus strip. Each point is the mean response of at least 8 measurements; vertical line show standard deviation of the mean (S.D.). The ordinate scale shows response as percentage of maximum contraction and the abscissa scale is the concentrations range 5.0×10^{-8} M - 10.0×10^{-5} M of acetylcholine. All points on the curves from all concentrations of the crude extract treated condition are significantly different from the control curve.(★ ——— ★). (P<0.001, Student's t-test)

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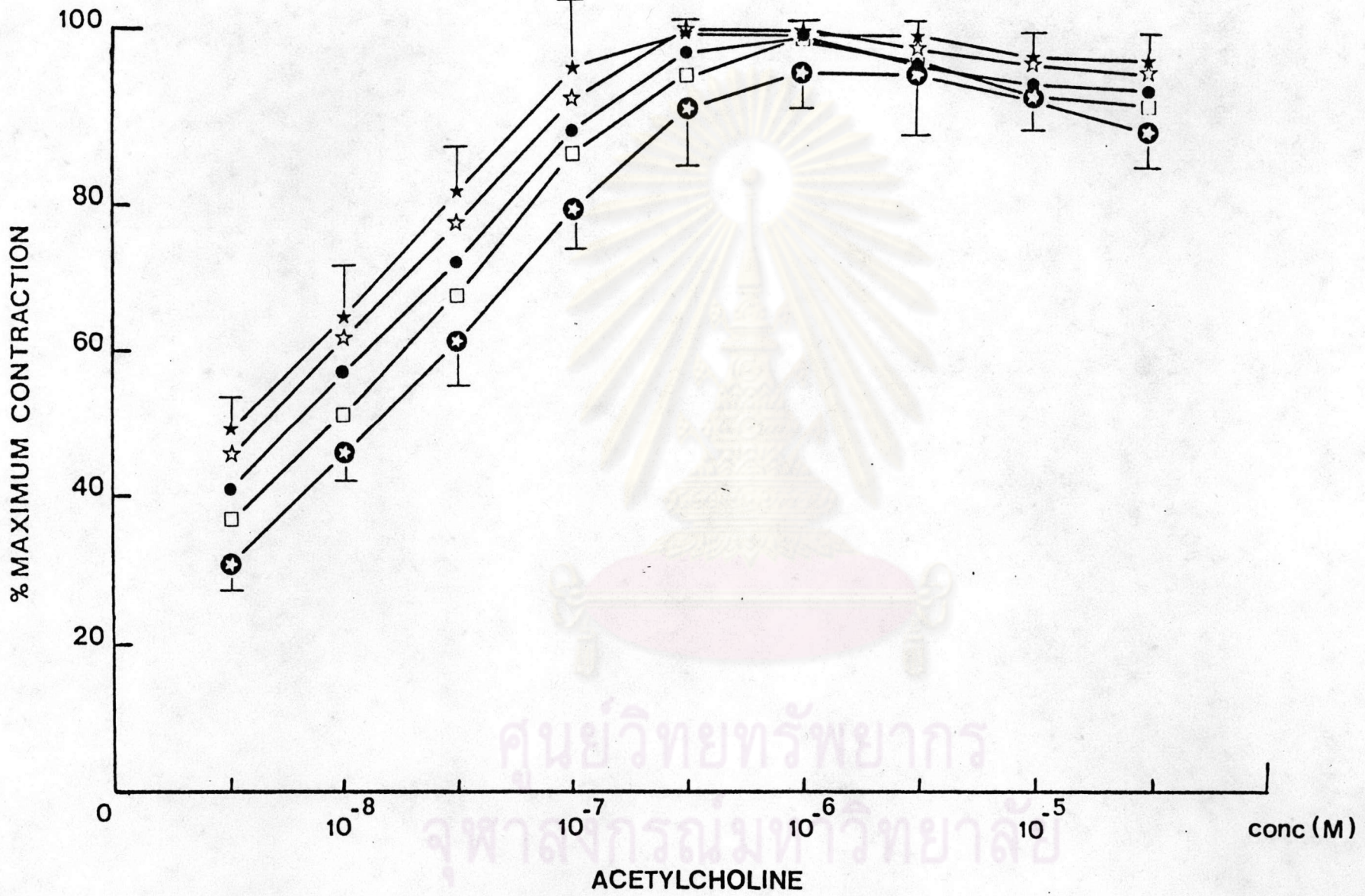


Fig 17

Fig 18 Cumulative concentration-response curves of histamine on tone of contraction of the isolated rat duodenum segment following preincubation for 10 min with the crude extract from C. citratus at concentration 0.25 g/ml; 0.2 ml (□), 0.25 g/ml; 0.3 ml (●), 1.00 g/ml; 0.2 ml (☆) and 1.00 g/ml; 0.3 ml (★). Histamine induced sustained contraction of the isolated rat stomach fundus strip. Each point is the mean response of at least 8 measurements; vertical line show standard deviation of the mean (S.D.). The ordinate scale shows response as percentage of maximum contraction and the abscissa scale is the concentrations range 5.0×10^{-8} M - 10.0×10^{-4} M of histamine. All points on the curves from all concentrations of the crude extract treated condition are significantly different from the control curve. (★—★). (P<0.001, Student's t-test)

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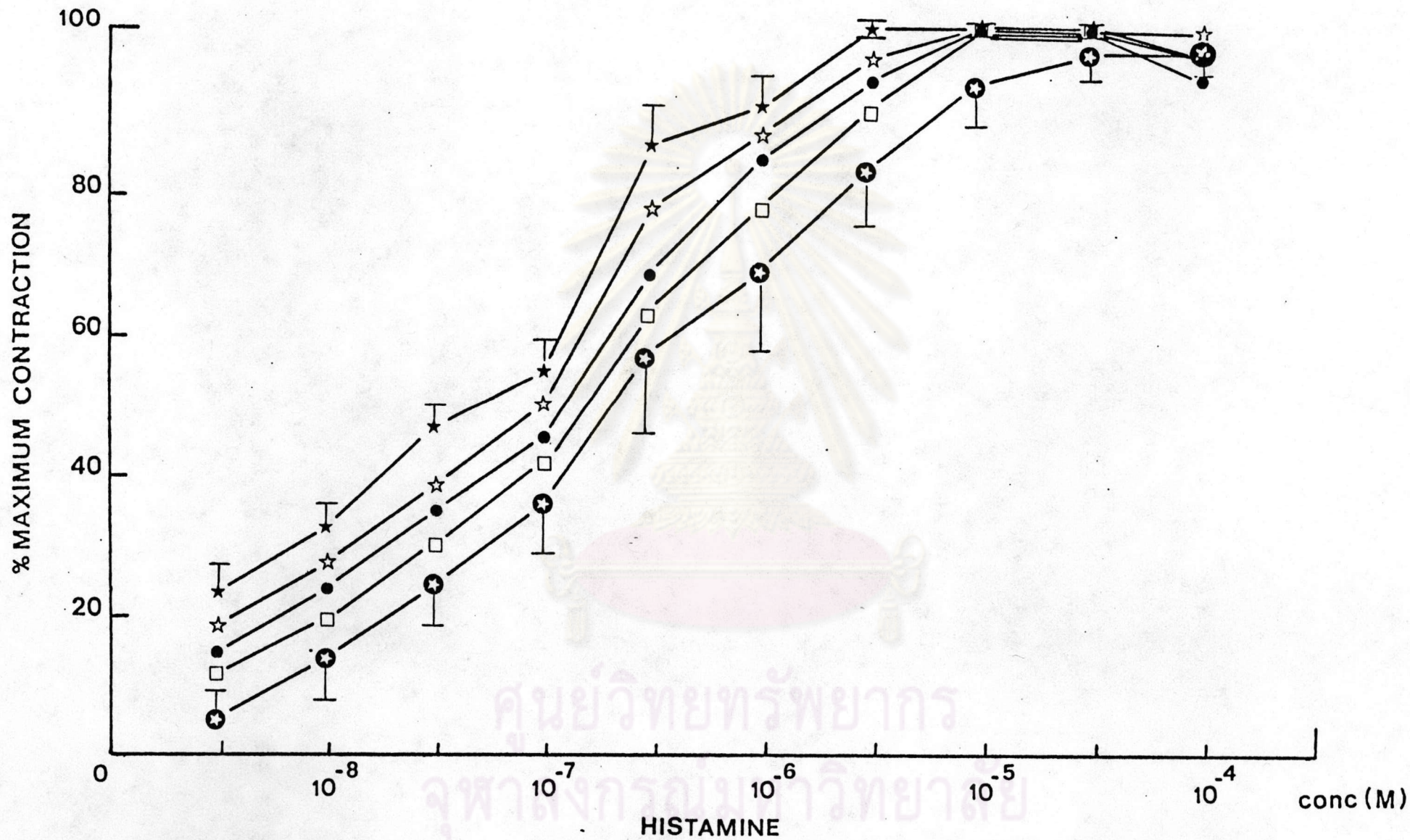


Fig 18

Fig 19 Cumulative concentration-response curves of 5-HT on tone of contraction of the isolated rat duodenum segment following preincubation for 10 min with the crude extract from C. citratus at concentration 0.25 g/ml; 0.2 ml (□), 0.25 g/ml; 0.3 ml (●), 1.00 g/ml; 0.2 ml (☆) and 1.00 g/ml; 0.3 ml (★). 5-HT induced sustained contraction of the isolated rat stomach fundus strip. Each point is the mean response of at least 8 measurements; vertical line show standard deviation of the mean (S.D.). The ordinate scale shows response as percentage of maximum contraction and the abscissa scale is the concentrations range 5.0×10^{-9} M - 10.0×10^{-5} M of 5-HT. All points on the curves from all concentrations of the crude extract treated condition are significantly different from the control curve. (★ ——— ★). (P<0.001, Student's t-test)

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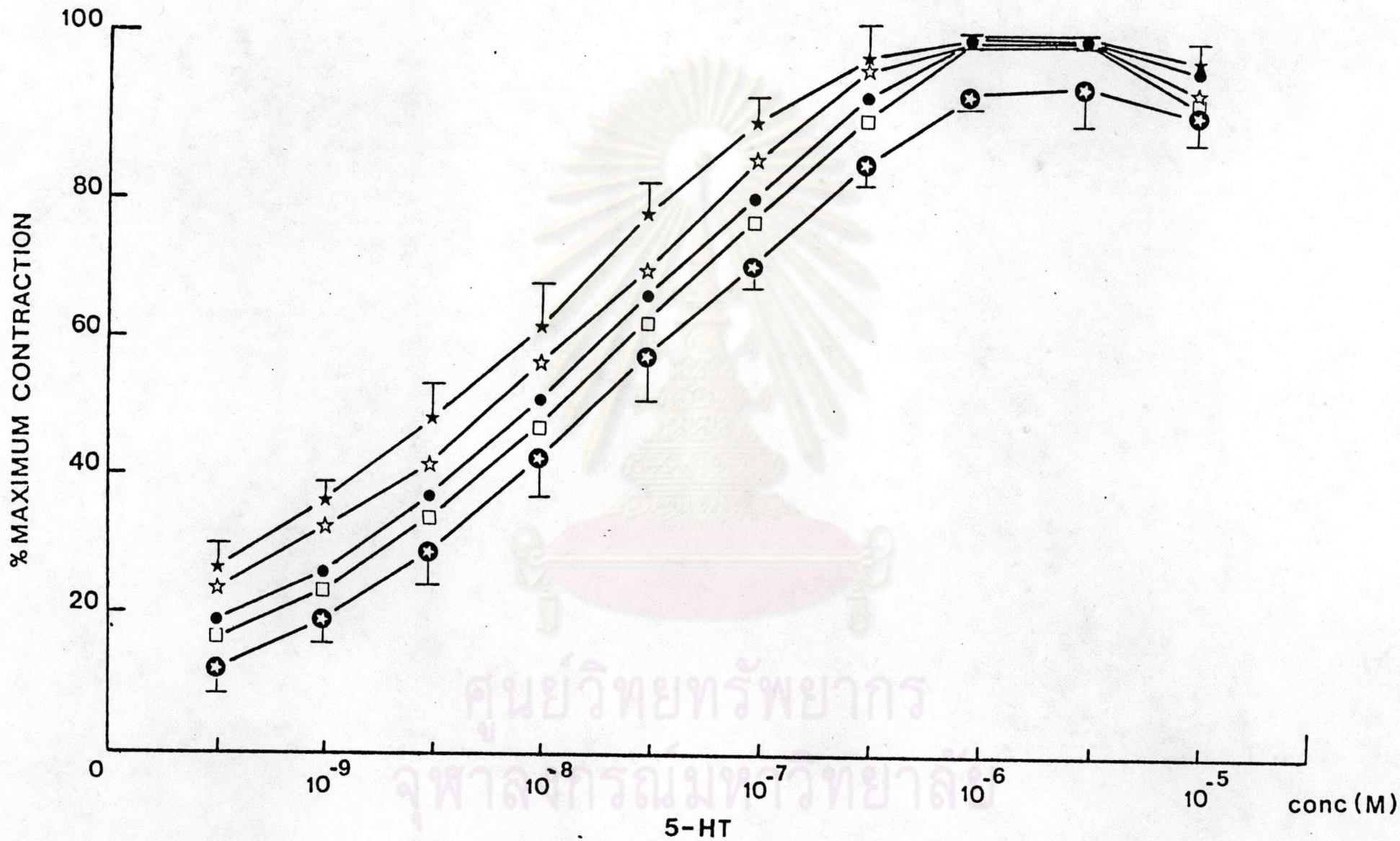


Fig 19

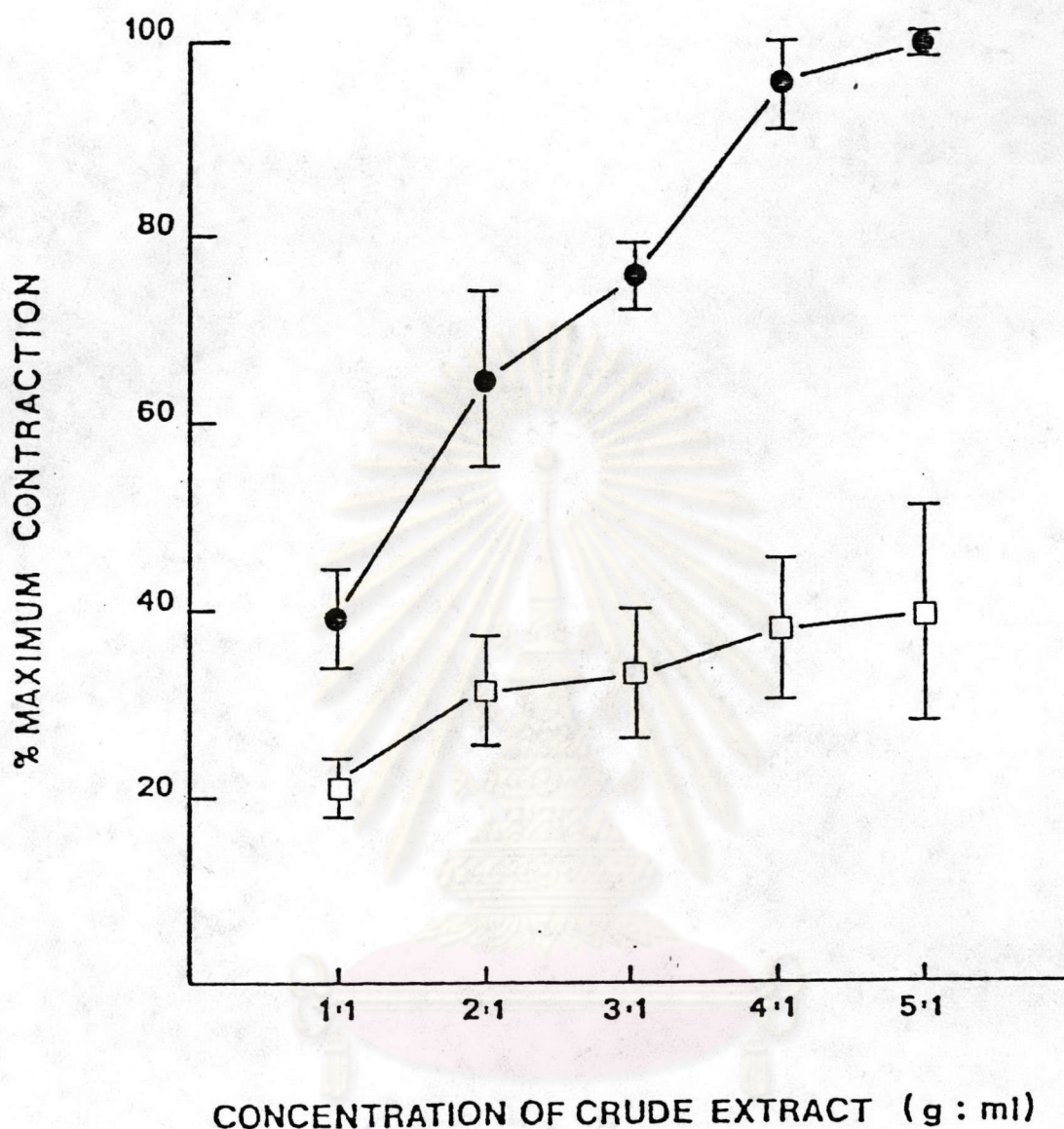


Fig 20 Cumulative concentration-response curves of the crude extract from C. citratus at concentrations range 1:1 - 5:1 (g:ml) on tone of contraction of the isolated rat duodenum segment in the absence (●) and presence of atropine at concentration 75 µg/ml (□), which preincubated for 10 min. All concentrations of the crude extract induced sustained contraction of the isolated this tissue, incubation of the tissue with atropine produced significantly block of the contractile response of the crude extracts. ($p < 0.005$) ($n=8$)

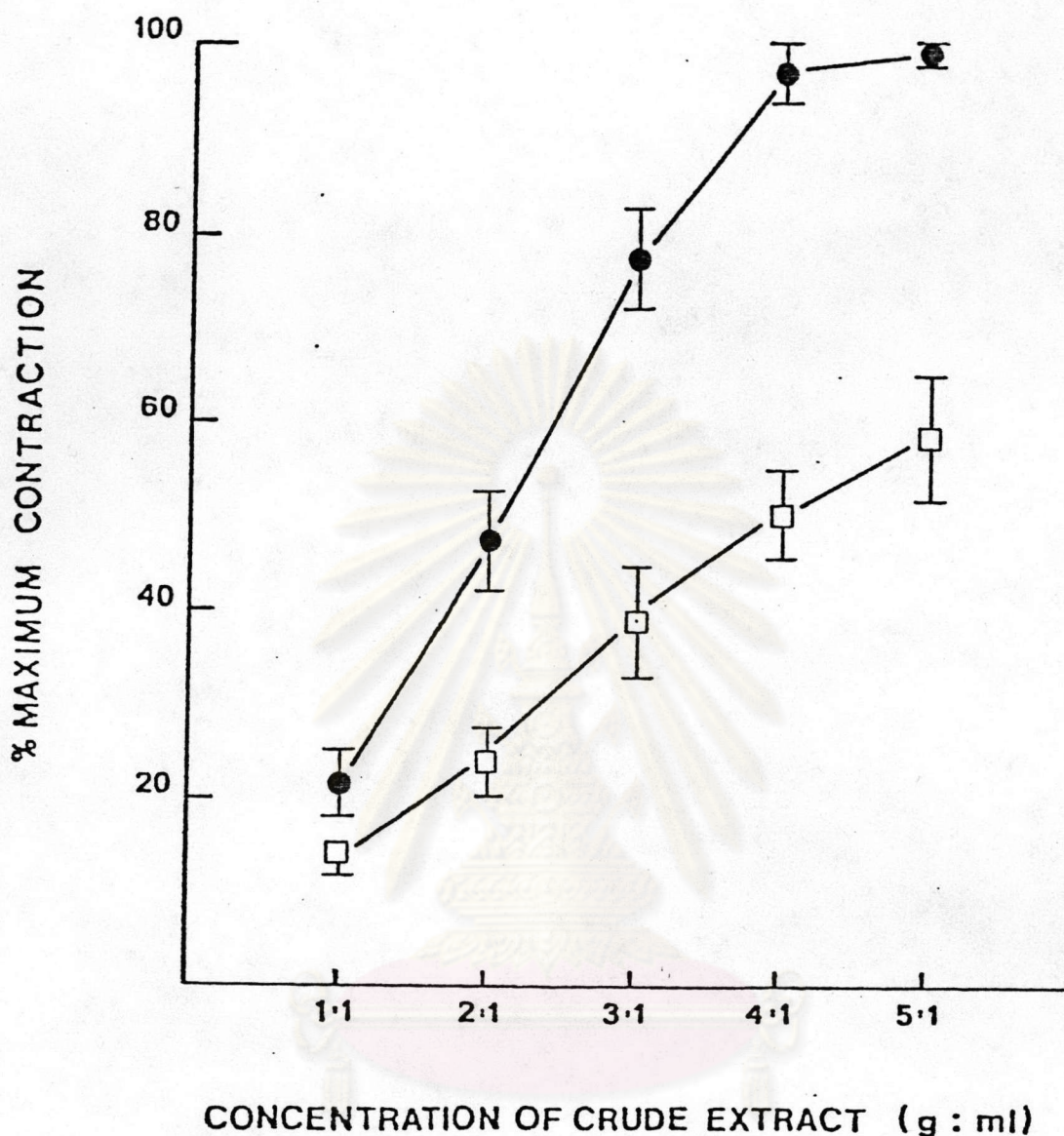


Fig 21 Cumulative concentration-response curves of the crude extract from *C. citratus* at concentrations range 1:1 - 5:1 (g:ml) on tone of contraction of the isolated rat duodenum segment in the absence (●) and presence of chlorpheniramine at concentration 75 µg/ml (□), which preincubated for 10 min. All concentrations of the crude extract induced sustained contraction of the isolated this tissue, incubation of the tissue with chlorpheniramine produced significantly block of the contractile response of the crude extracts. ($p < 0.010$) ($n=8$)

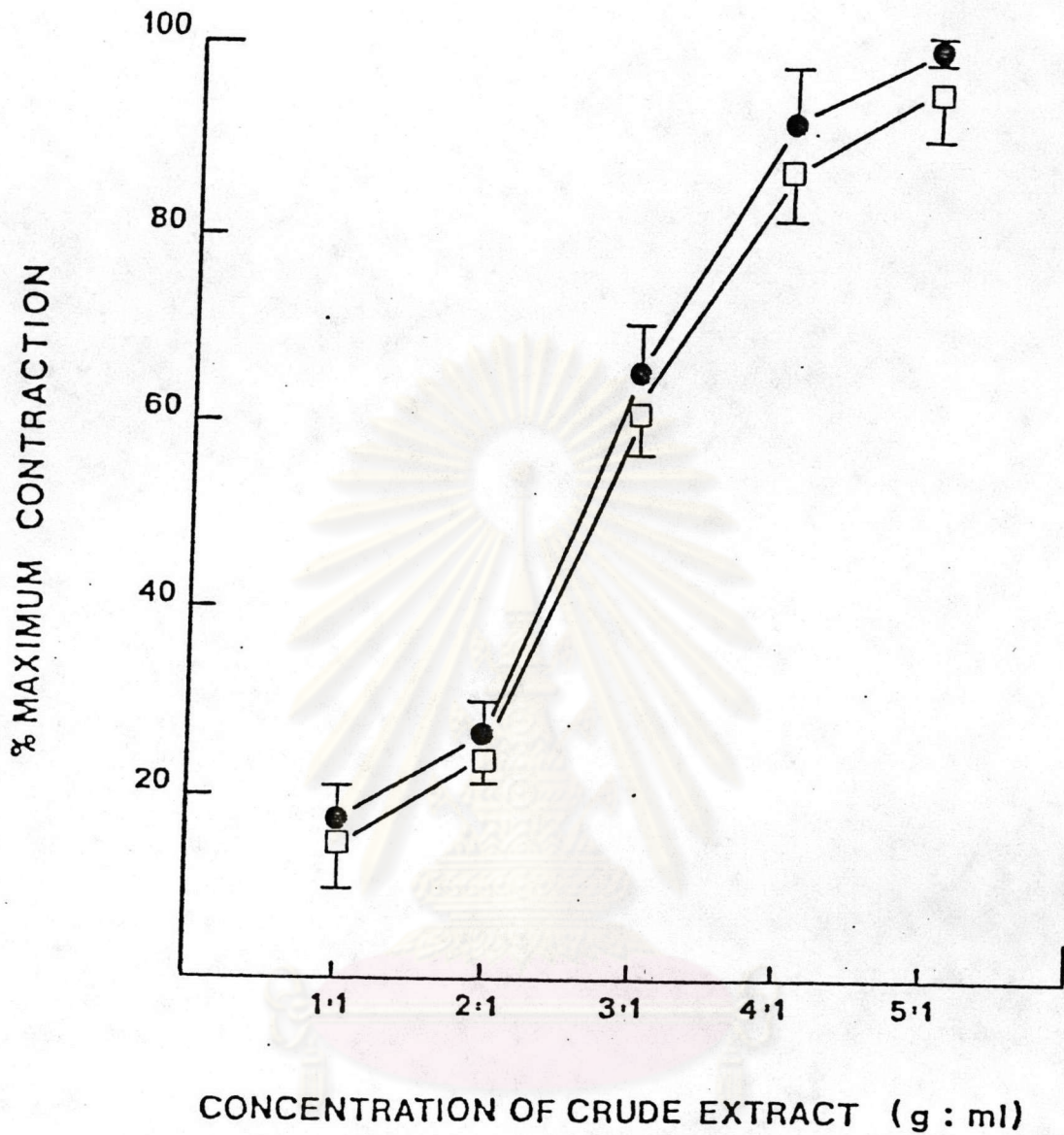


Fig 22 Cumulative concentration-response curves of the crude extract from C. citratus at concentrations range 1:1 - 5:1 (g:ml) on tone of contraction of the isolated rat duodenum segment in the absence (●) and presence of cimetidine at concentration 75 µg/ml (□), which preincubated for 10 min. All concentrations of the crude extract induced sustained contraction of the isolated this tissue, incubation of the tissue with cimetidine produced non-significantly block of the contractile response of the crude extracts. (n=8)

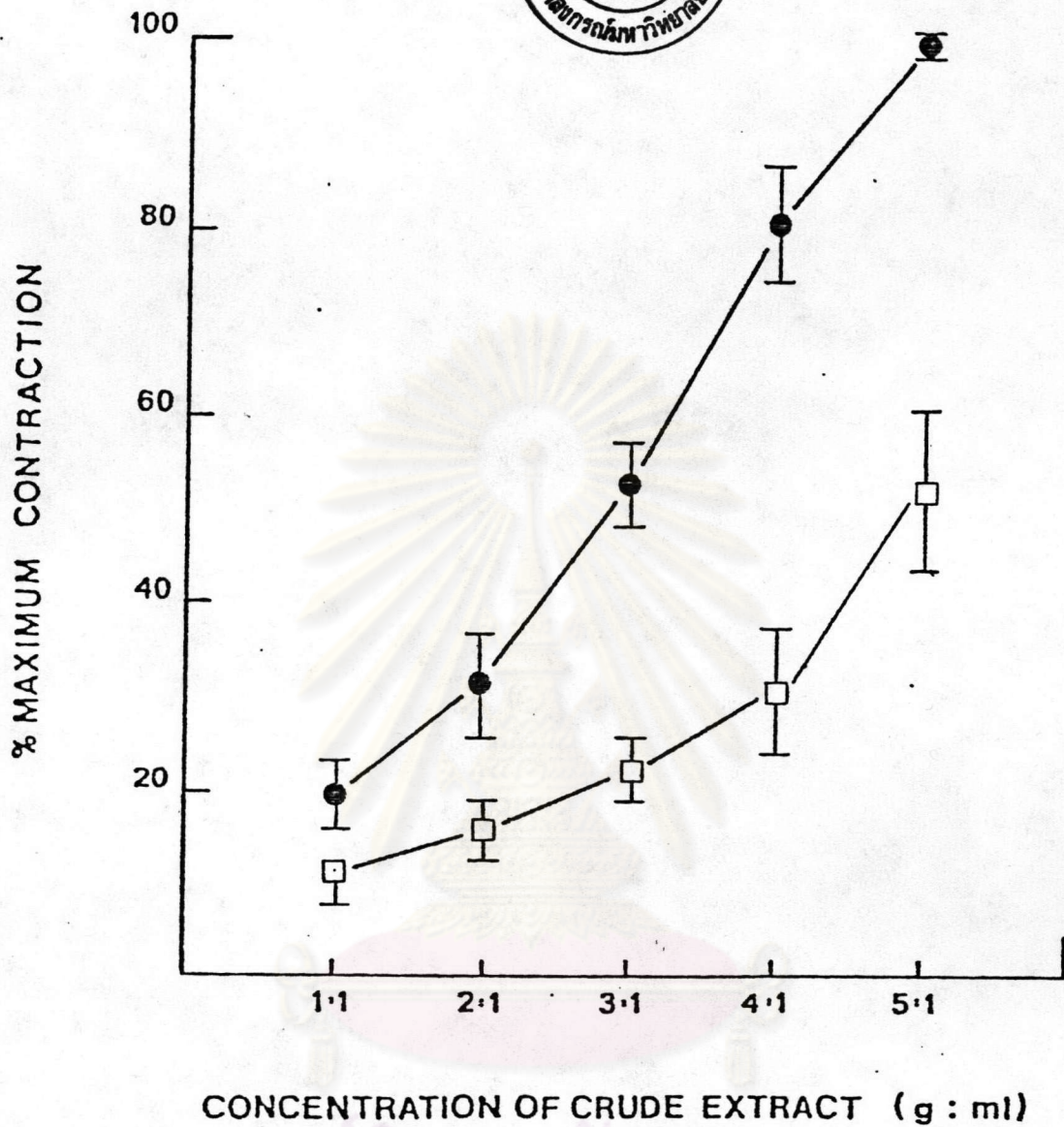


Fig 23 Cumulative concentration-response curves of the crude extract from *C. citratus* at concentrations range 1:1 - 5:1 (g:ml) on tone of contraction of the isolated rat duodenum segment in the absence (●) and presence of chlorpheniramine and cimetidine at concentration 75 μ g/ml (□), which preincubated for 10 min. All concentrations of the crude extract induced sustained contraction of the isolated this tissue, incubation of the tissue with chlorpheniramine and cimetidine produced significantly block of the contractile response of the crude extracts. ($p < 0.010$) (n=8)

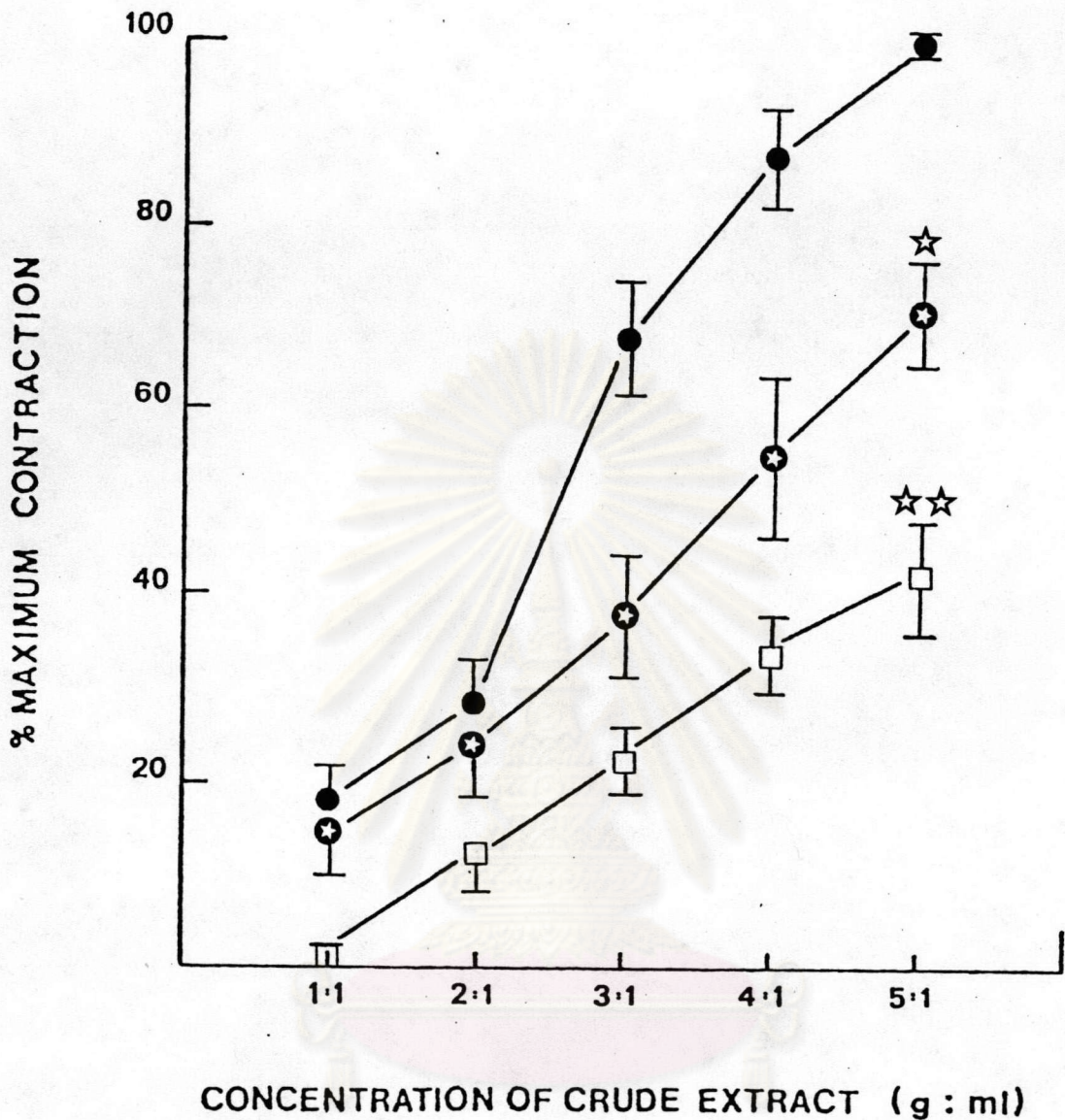


Fig 24 Cumulative concentration-response curves of the crude extract from *C. citratus* at concentrations range 1:1 - 5:1 (g:ml) on tone of contraction of the isolated rat duodenum segment in the absence (●) and presence of cyproheptadine (75 μ g/ml) with 70% ethanol (□), and 70% ethanol (●*), which preincubated for 10 min. All concentrations of the crude extract induced sustained contraction of the isolated this tissue, incubation of the tissue with cyproheptadine and 70% ethanol produced significantly block of the contractile response of the crude extracts (☆; ☆☆, $p < 0.025$; $p < 0.010$). (n=10)

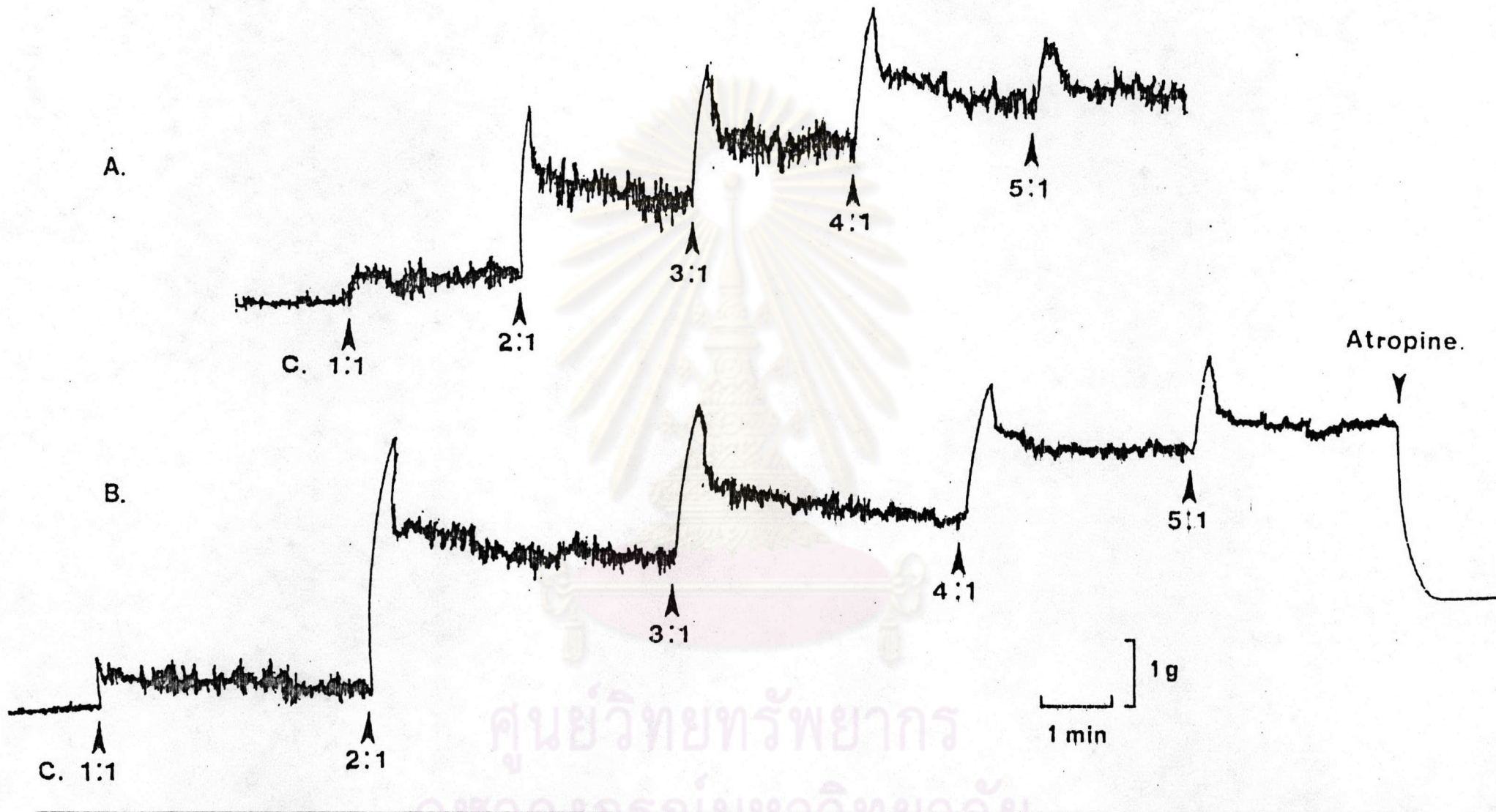


Fig 25 Trace of cumulative dose-response relationship of the crude extract from *C. citratus* at concentrations range 1:1 - 5:1 (g:ml); 0.3 ml on the isolated guinea-pig ileum segment. The record shows the crude extracts caused increase tone of contraction (A. and B.) and this effect blocked by 75 µg/ml of atropine (B.)

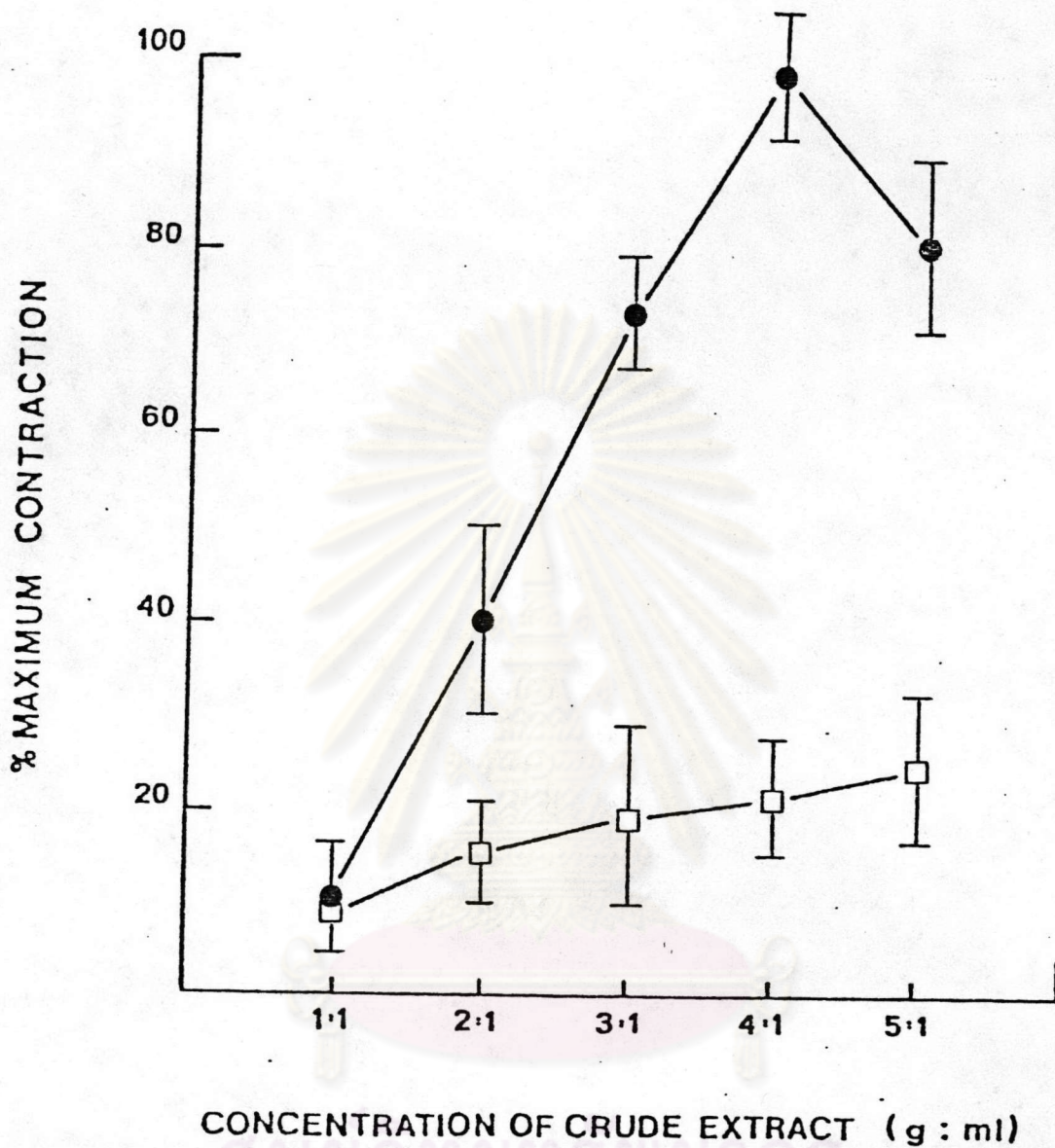


Fig 26 The effect of the crude extract from *C. citratus* at concentrations range 1:1 - 5:1 (g:ml) on tone of contraction of the isolated guinea-pig ileum segment in a cumulative dose regimen (●) and in washed out each dose every 5 min of treatment (□). The effect of all concentrations of the crude extract on washed out concentration-response curve is lesser than cumulative concentration-response curve significantly on tone of contraction of the isolated guinea-pig ileum segment. ($p < 0.001$)

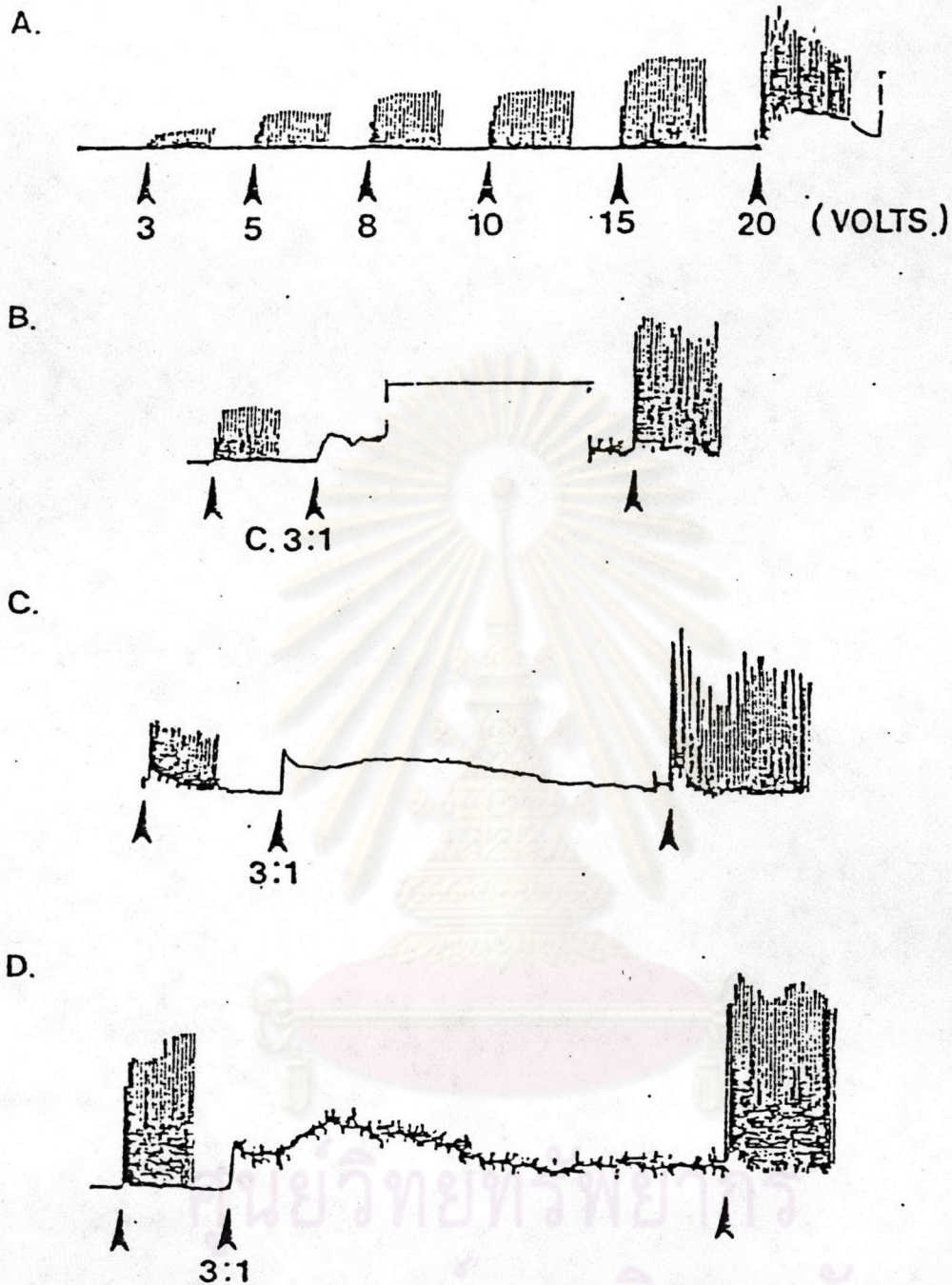


Fig 27 Trace shows the effect of coaxial electrical stimulating at varying voltages on amplitude (mm) contraction of the isolated guinea-pig ileum segment (A), and compare the effect of coaxial electrical stimulating at 3V. (B), 5V. (C) and 10V. (D) which was indicated (\blacktriangle) before and after treat with the crude extract at concentration 3:1 (g:ml) for 10 minutes. The crude extract caused significantly increase amplitude of contraction of the isolated guinea-pig ileum segment induced by coaxial electrical stimulation. (P.<0.001)

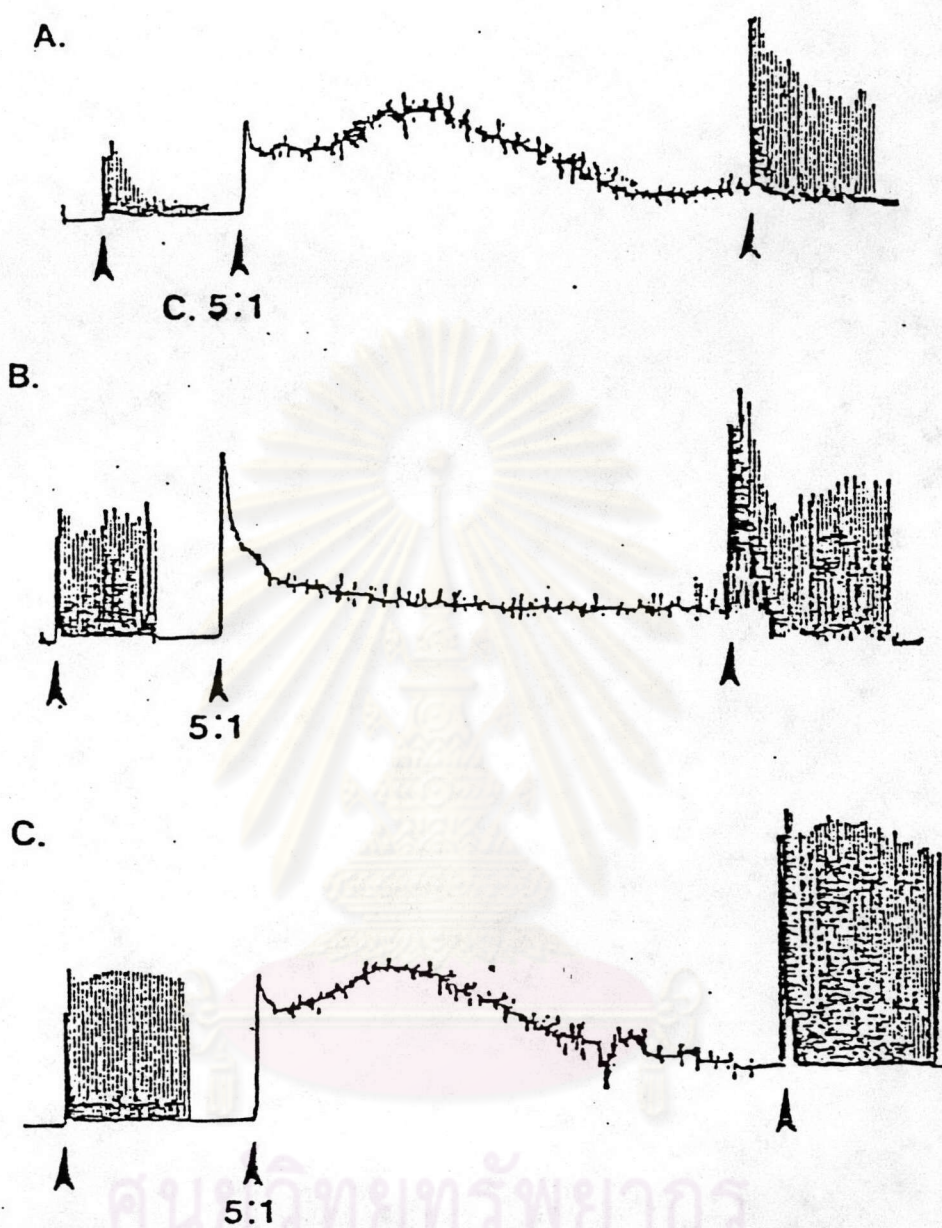


Fig 28

Comparisms the effect of coaxial electrical stimulating at 3V. (A), 5V (B) and 10V. (C) which was indicated (▲) before and after treat with the crude extract at concentration 5:1 (g:ml) for 10 minutes on amplitude of contraction of the isolated guinea-pig ileum segment. (continue from Fig 27.)

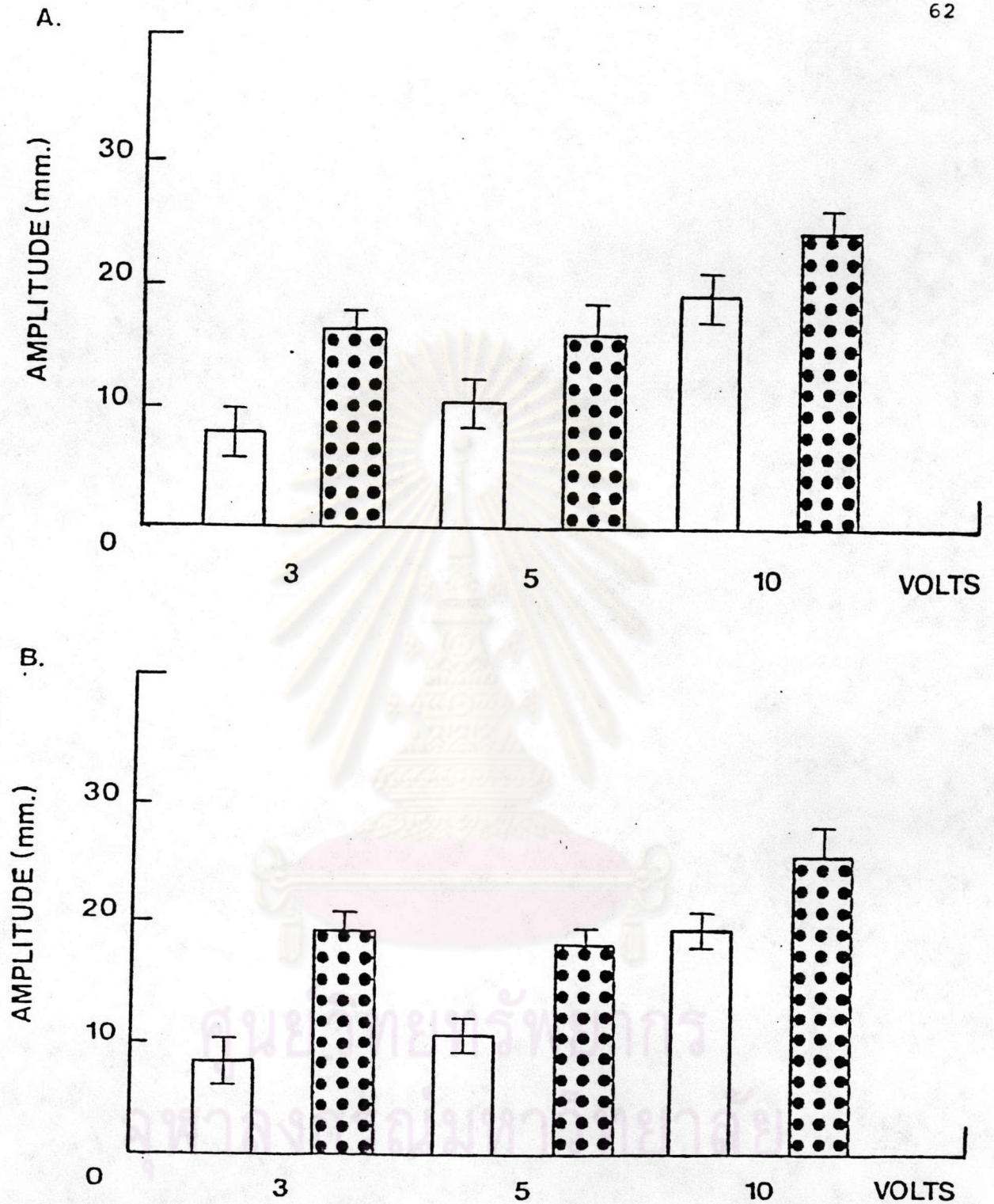


Fig 29 Shows bar graphs on amplitude of contraction of the isolated guinea-pig ileum segment induced by coaxial electrical stimulating at 3V., 5V. and 10V. before (\square) and after (\bullet) treat with the crude extract at concentration 3:1 (A) and 5:1 (B) respectively (g:ml). Each bar graph is the mean response of at least 10 measurement and vertical lines show S.D. of mean.

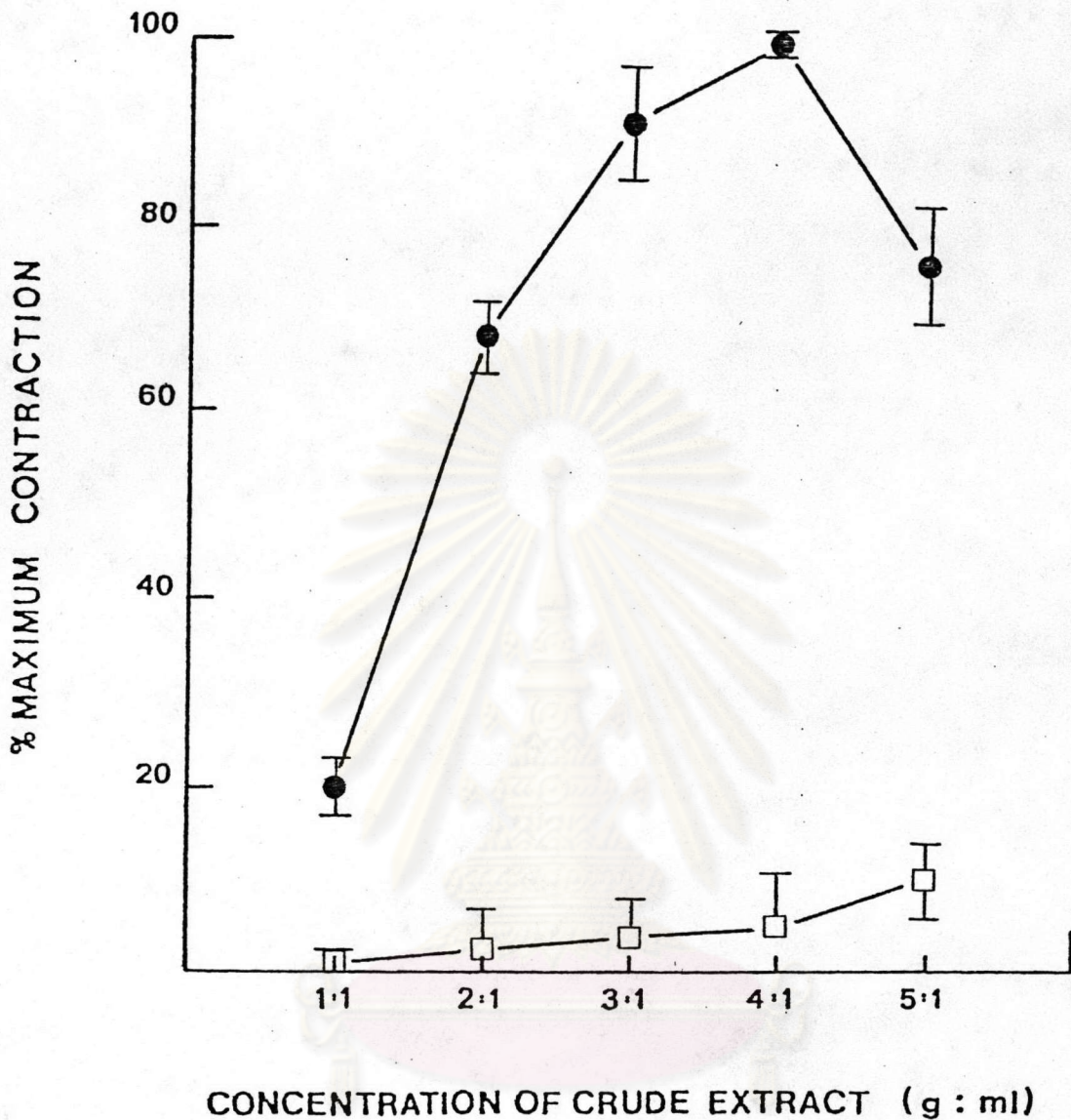


Fig 30 Cumulative concentration-response curves of the crude extract from C. citratus at concentrations range 1:1 - 5:1 (g:ml) on tone of contraction of the isolated guinea-pig ileum segment in the absence (●) and presence of atropine at concentration 75 µg/ml (□), which preincubated for 10 min. All concentrations of the crude extract induced sustained contraction of the isolated this tissue, incubation of the tissue with atropine produced significantly block of the contractile response of the crude extracts. ($p < 0.005$) (n=8)

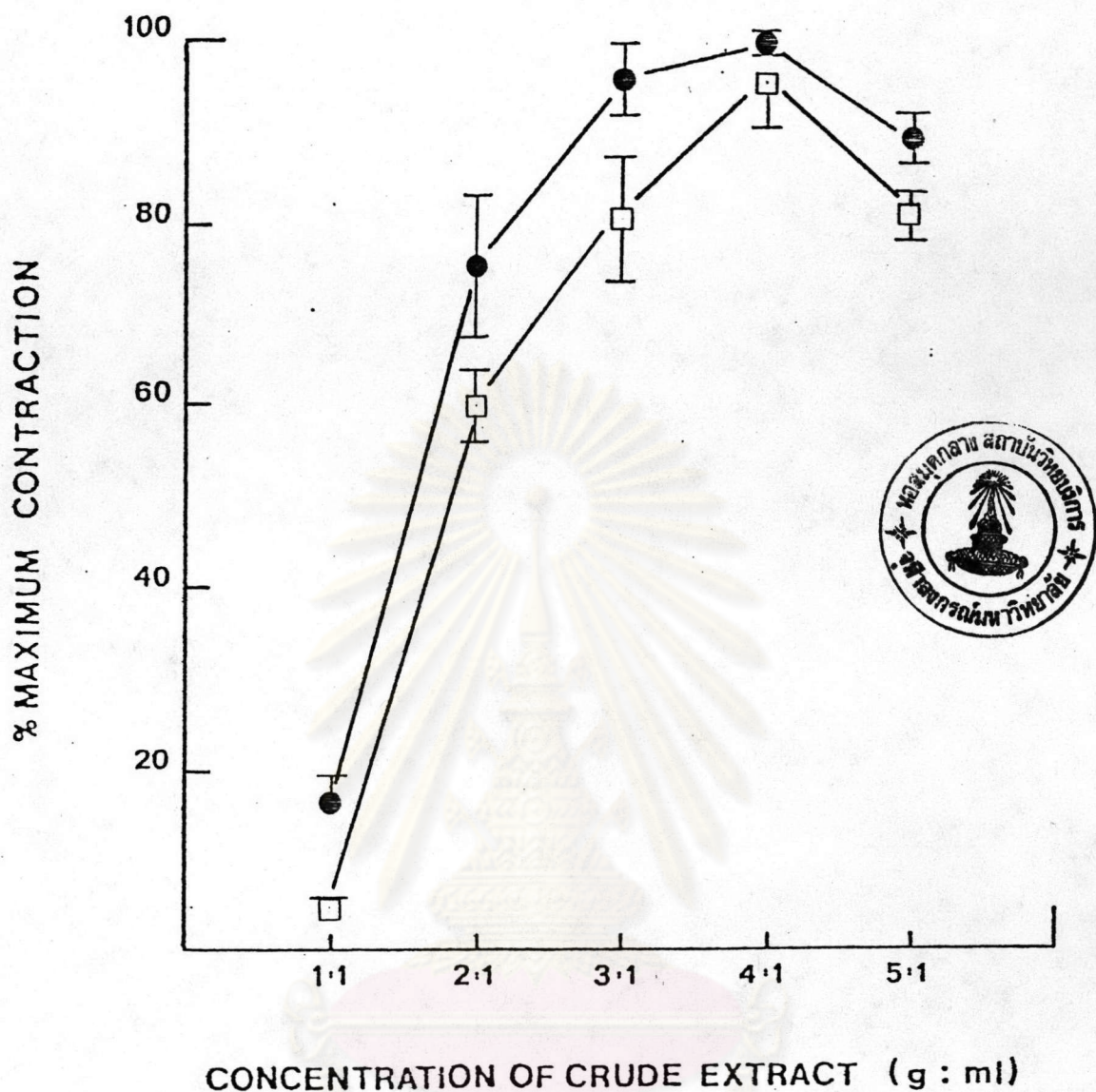


Fig 31 Cumulative concentration-response curves of the crude extract from *C. citratus* at concentrations range 1:1 - 5:1 (g:ml) on tone of contraction of the isolated guinea-pig ileum segment in the absence (●) and presence of chlorpheniramine at concentration 75 µg/ml (□), which preincubated for 10 min. All concentrations of the crude extract induced sustained contraction of the isolated this tissue, incubation of the tissue with chlorpheniramine produced significantly block of the contractile response of the crude extracts. ($p < 0.010$) ($n=8$)

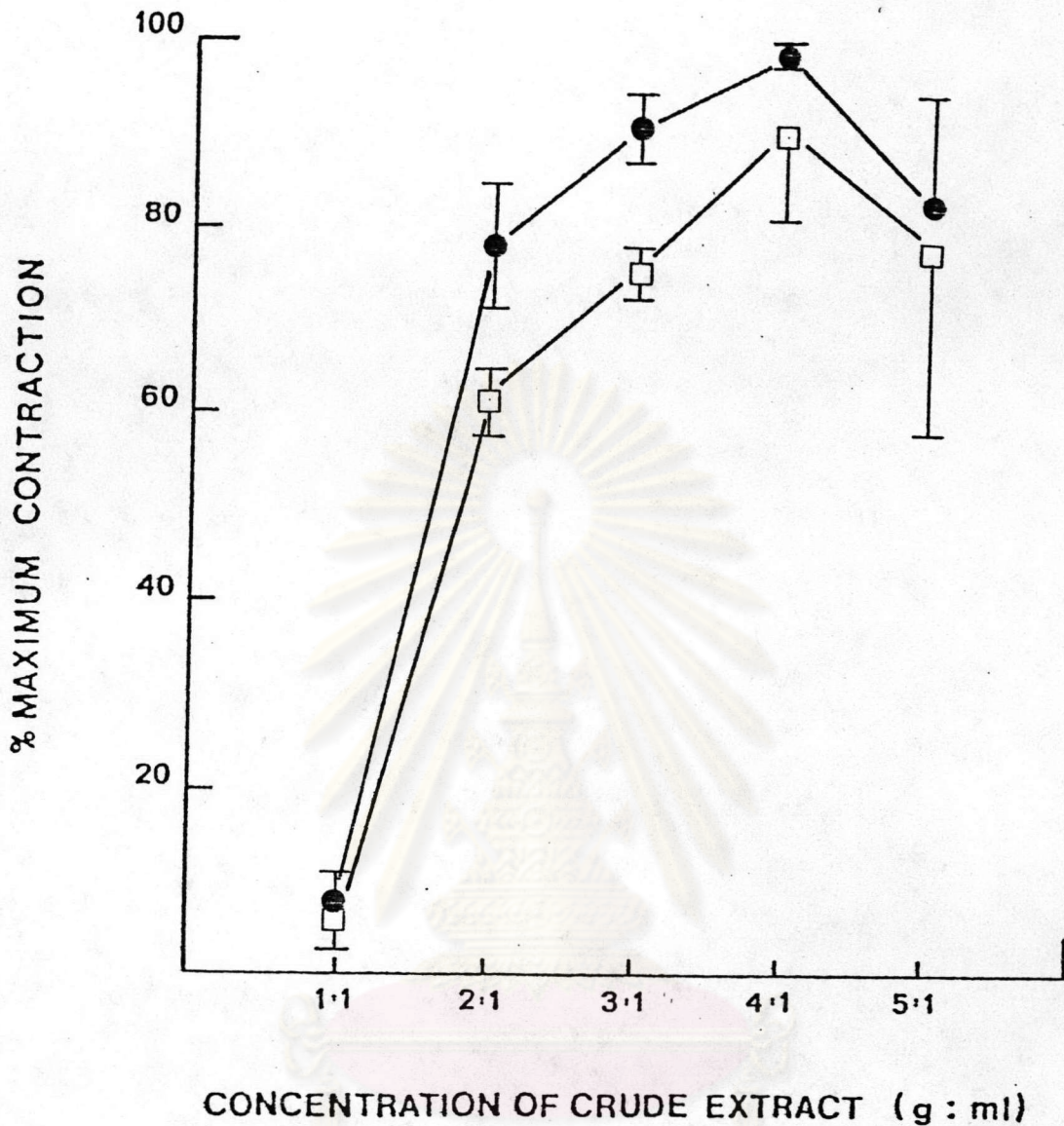


Fig 32 Cumulative concentration-response curves of the crude extract from *C. citratus* at concentrations range 1:1 - 5:1 (g:ml) on tone of contraction of the isolated guinea-pig ileum segment in the absence (●) and presence of cimetidine at concentration 75 µg/ml (□), which preincubated for 10 min. All concentrations of the crude extract induced sustained contraction of the isolated this tissue, incubation of the tissue with cimetidine produced non-significant block of the contractile response of the crude extracts. (n=8)

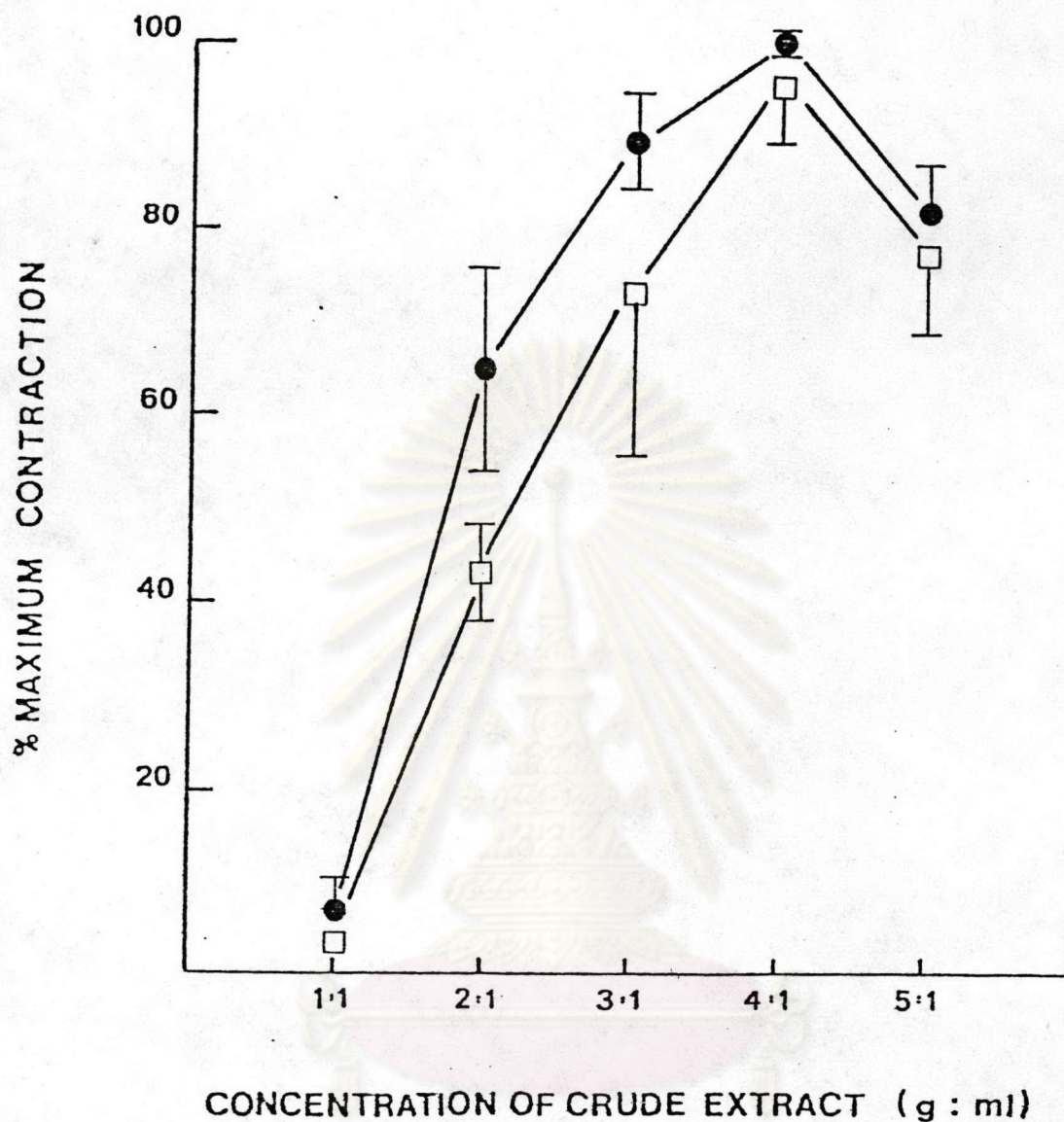


Fig 33 Cumulative concentration-response curves of the crude extract from *C. citratus* at concentrations range 1:1 - 5:1 (g:ml) on tone of contraction of the isolated guinea-pig ileum segment in the absence (●) and presence of chlorpheniramine and cimetidine at concentration 75 $\mu\text{g/ml}$ (□), which preincubated for 10 min. All concentrations of the crude extract induced sustained contraction of the isolated this tissue, incubation of the tissue with chlorpheniramine and cimetidine produced significantly block of the contractile response of the crude extracts. ($p < 0.010$) (n=8)

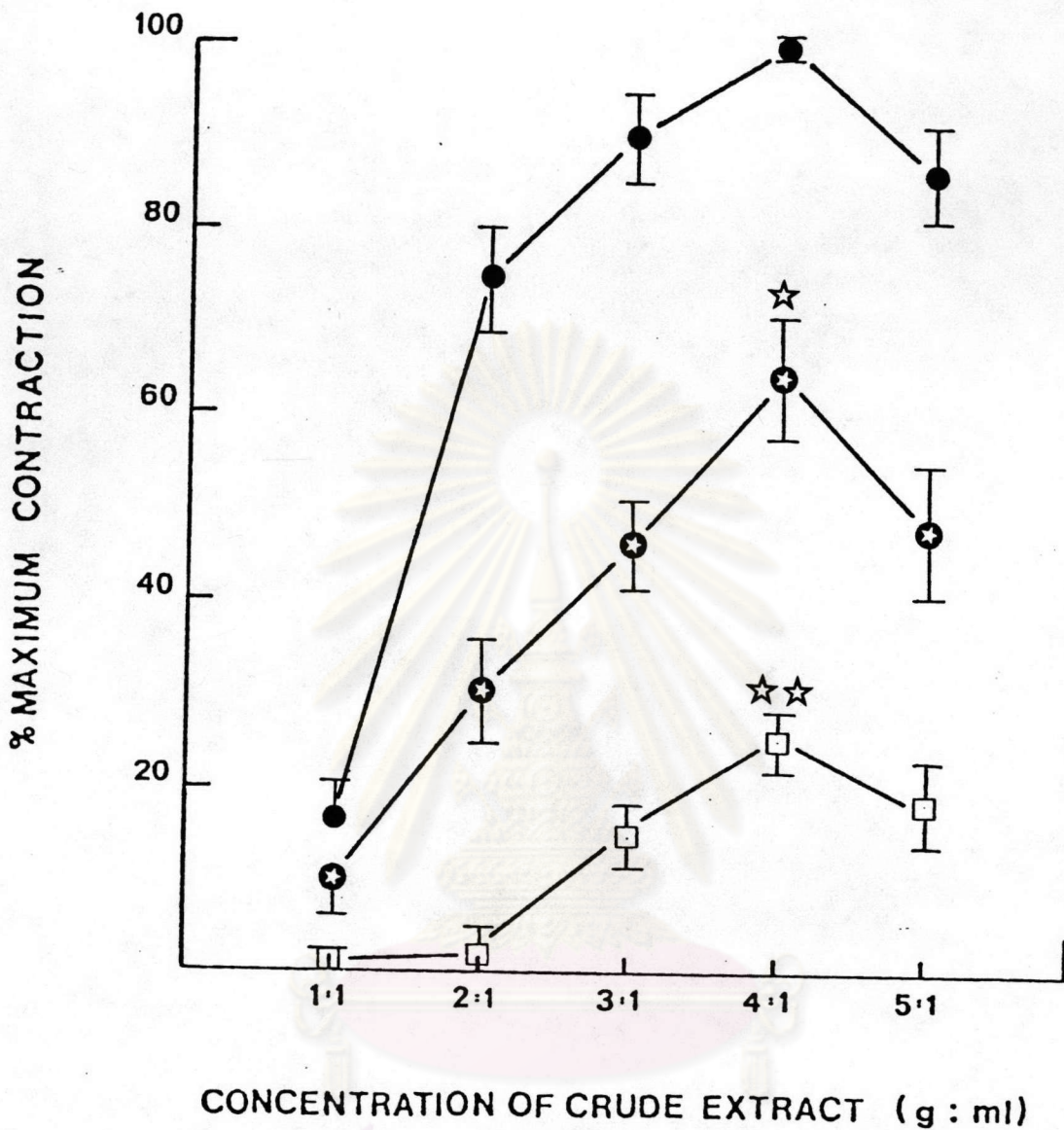


Fig 34 Cumulative concentration-response curves of the crude extract from *C. citratus* at concentrations range 1:1 - 5:1 (g:ml) on tone of contraction of the isolated guinea-pig ileum in the absence (●) and presence of cyproheptadine (75 μ g/ml) with 70% ethanol (□), and 70% ethanol (★), which preincubated for 10 min. All concentrations of the crude extract induced sustained contraction of the isolated this tissue, incubation of the tissue with cyproheptadine and 70% ethanol produced significantly block of the contractile response of the crude extracts (★ $p < 0.005$; ★★ $p < 0.005$). (n=10)

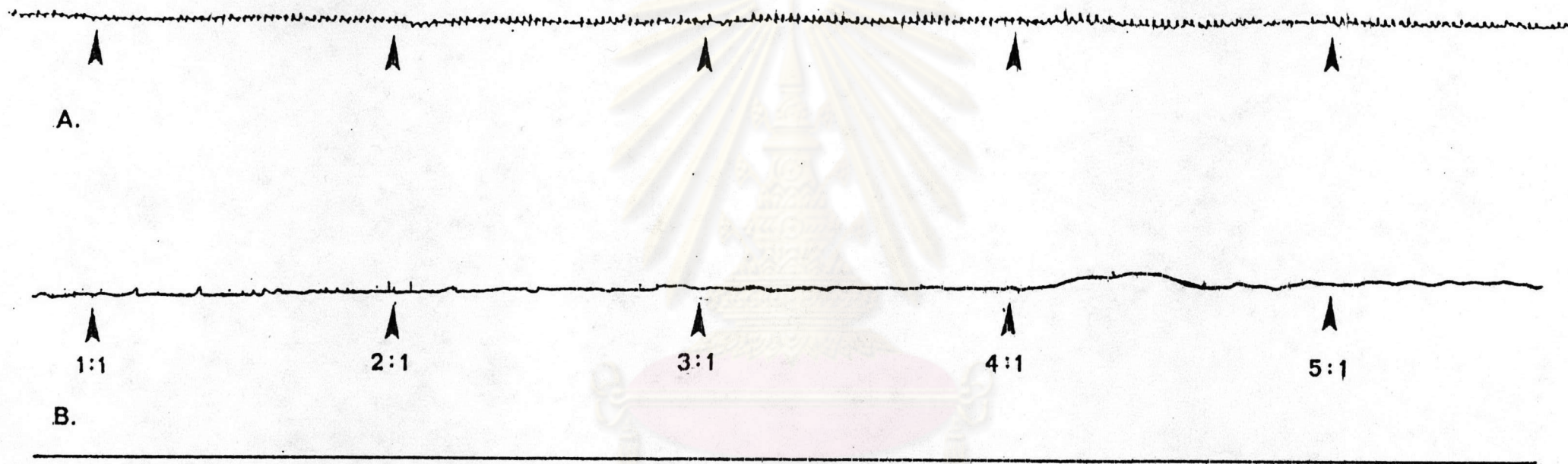


Fig 35 Trace of cumulative dose-response relationship of the crude extract from C. citratus at concentrations range 1:1 -5:1 (g:ml); 0.3 ml on tone of contraction on mucosal (B.) and serosal side (A.) of the isolated mouse stomach. The record shows the crude extracts caused increase tone of contraction on mucosal side but no effect on serosal side of the isolated mouse stomach significantly.

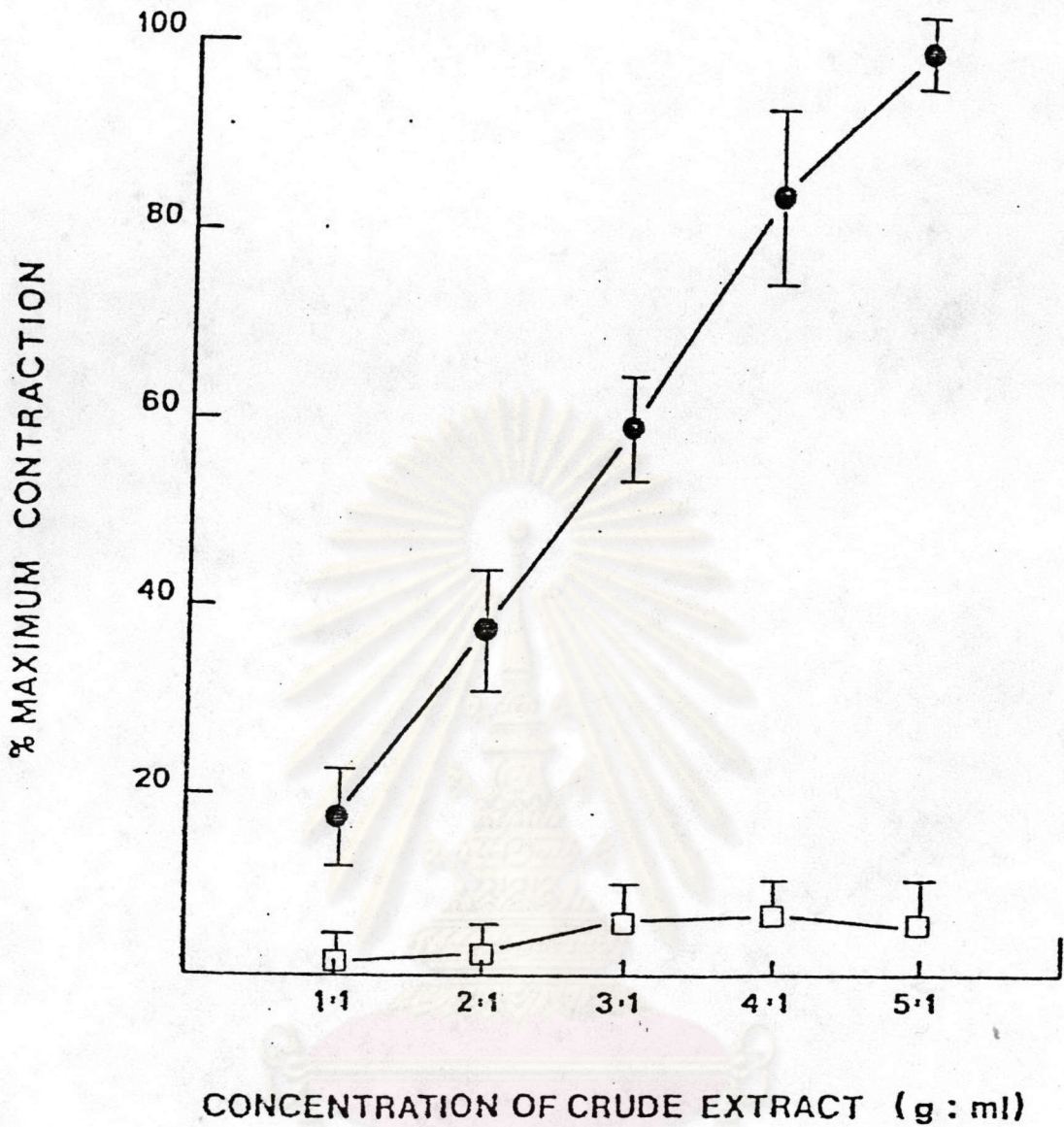


Fig 36

Cumulative concentration-response curves of the crude extract from *C. citratus* at concentrations range 1:1-5:1 (g:ml) on tone of contraction on mucosal side (●) and serosal side (□) of the isolated mouse stomach. All concentrations of the crude extract caused significantly increase tone of contraction on mucosal side, but no effect on serosal side when compared with mucosal side of the isolated mouse stomach. (P.<0.0010)