CHAPTER 4

RESULTS

Extraction of medicinal plants

The yield and texture of crude methanolic extract from each medicinal plant are shown in Table 2

Table 2 Yield and texture of methanolic extract from each plant

Plant	Started material (g)	Texture	Color	Yield (g)
Piper longum	1,000	viscous oil	orange	228.40
Piper sarmentosum	471	semi-solid	dark green	21.34
Quercus infectoria	477.34	powder	brown	222.79

Section 1

1.1 Effects of *Piper longum*, *Piper sarmentosum* and *Quercus infectoria* on caecal amoebiasis in mice

The effects of crude extract of *P. longum*, *P. sarmentosum* and *Q. infectoria* against experimental caecal amoebiasis in mice are shown in Table 3. The crude extract of *P. longum* at the dose of 1000 mg/kg gave 100% cure rate, the average score of caecal content and wall were 0. At the dose of 500 mg/kg, 250 mg/kg and 125 mg/kg, the average score of caecal content and wall were 0, 0 and 0.4, with the cure rate of 93%, 46% and 0%, respectively. The methanolic extract of *P. sarmentosum* showed 40% cure at the dose of 1000 mg/kg, the average caecal score of content and wall were 0.2. At the dose of 500 mg/kg, 250 mg/kg and 125 mg/kg, the average score of caecal content were 1.4, 2, and 2, the average score of caecal walls were 1.26, 2 and 2 and the cure rate were 0%, 0% and 0%, respectively. The methanolic extract of *Q. infectoria* showed 26% cure at the dose of 1000 mg/kg, the average caecal score of content and wall were 0.01. At the dose of 500

mg/kg, 250 mg/kg and 125 mg/kg, the average score of caecal content were 0.25, 0.62 and 1.06, the average caecal score of walls were 0.25, 0.62 and 0.93 and the cure rate were 26%, 13% and 0%, respectively. Metronidazole reduced the severity of infection by 60% at the dose of 62.5 mg/kg, the average score of caecal content and wall were 0.06. While a complete cure was observed when the dose of metronidazole was doubled to 125 mg/kg, with which average score of caecal content and wall were 0. The pooled controls were a total of 20 mice and all of them were positive for amoebae at the time of sacrifice. The control animals generally had score of caecal content and wall ranging between 2-3 and the average of these values were 2.55 and 2.40, respectively.

Section 2

2.1 Effects of Plants extracts, loperamide, verapamil and the receptor antagonists on the contractile responses of rat or guinea-pig ileum to acetylcholine, serotonin and histamine

2.1.1 Effects of plant extracts

All extracts, *P. longum* (0.01-1 mg/ml), *P. sarmentosum* (0.01-1 mg/ml) and *Q. infectoria* (0.1-10 mg/ml) exerted significant inhibitory effects on the contractions of rat ileum induced by acetylcholine (10 μM) or serotonin (3 μM) as well as on the guinea-pig ileal contraction-induced by histamine (1 μM) in the concentration-dependent manners. The contractions were almost completely or completely abolished by the highest concentration of the extract used in this study (see representative trace in Figure 17 - 19). Data were summarized in Figure 20 - 22.

Table 3 Effect of methanolic extracts of *P. longum*, *P. Sarmentosum* and *Q. infectoria* on caecal amoebiasis of mice

Test materials	Dose (mg/kg)	No. of mice (cleared/treated) (% cured)	Average caecal score* (range)	
			Contents	Walls
P. longum	125	0/15 (0)	0.4 (0-1)	0.4 (0-1)
	250	7/15 (46)	0 (0-0)	0 (0-0)
	500	14/15 (93)	0 (0-0)	0 (0-0)
	1,000	15/15 (100)	0 (0-0)	0 (0-0)
P. sarmentosum	125	0/15 (0)	2 (2-2)	2 (2-2)
	250	0/15 (0)	2 (2-2)	2 (2-2)
	500	0/15 (0)	1.4 (0-2)	1.26 (0-2)
	1,000	6/15 (40)	0.2 (0-1)	0.2 (0-1)
Q. infectoria	125	0/15 (0)	1.06 (0-2)	0.93 (0-3)
	250	2/15 (13)	0.62 (0-2)	0.62 (0-2)
	500	4/15 (26)	0.25 (0-2)	0.25 (0-2)
	1,000	4/15 (26)	0.01 (0-2)	0.01 (0-2)
Metronidazole	62.5	9/15 (60)	0.06 (0-1)	0.06 (0-1)
	125	15/15 (100)	0 (0-0)	0 (0-0)
Untreated control		0/20 (0)	2.55 (2-3)	2.4 (2-3)

*Caecal scores were graded upon the following criteria (Neal, 1951)

Content
- Normal 0
- Slightly less solid than normal 1
- Slightly mucoid 2
- Mucoid, some solid matter present 3
- No solid matter, white or yellow mucus only 4

Wall
- Normal 0
- Slight thickening 1
- Marked local thickening and contraction 2
- Extensive thickening and contraction 3
- Caecum shapeless, extensive ulceration with abscess formation 4

Table 4 showed the IC_{50} values of the spasmolytic effects of the three plant extracts. The IC_{50} values calculated for *P. sarmentosum* and *P. longum* on the inhibition of acetylcholine and histamine-induced ileal contractions did not differ significantly ($p>0.05$) but each of them was significantly different ($p<0.05$) from that of *Q. infectoria.* The relative potency (based on concentration in μg/ml basis) of *P. sarmentosum* and *P. longum* were about 4 – 8 times more potent than *Q. infectoria.* However, the IC_{50} values of the three plant extracts on the inhibition of serotonin-induced ileal contraction did not differ significantly.

2.1.2 Effects of the reference drug, loperamide

Loperamide (0.3 - 10 μg/ml) produced similar inhibitory effect to those of the plant extracts. The highest concentration of loperamide (10 μg/ml) completely abolished the contraction-induced by all spasmogens (Figure 17 - 19). The results showed that loperamide was a very potent spasmolytic agent and was more potent than *P. sarmentosum, P. longum* and *Q. infectoria* by 15 - 139, 18 - 187 and 96 - 1,380 times, respectively (Table 5). The concentrations-response lines of loperamide and the three plant extracts did not differ significantly from parallelism (Figure 17 - 19).

2.1.3 Effects of the calcium antagonist, verapamil

Verapamil at the concentration of 10^{-8}-10^{-4} M (0.0049 - 49 μg/ml), also caused a decrease in the contractions of the isolated rat and guinea-pig ileum-induced by acetylcholine (10 μM), serotonin (3 μM) and histamine (1 μM). The blockades were also concentration-related (Figure 17 - 19). As shown in Figure 20 - 22 and Table 4 and 5b, verapamil possessed a very potent spasmolytic effect similar to loperamide and their IC_{50} values for the inhibitory effects of all spasmogens were not significantly different.

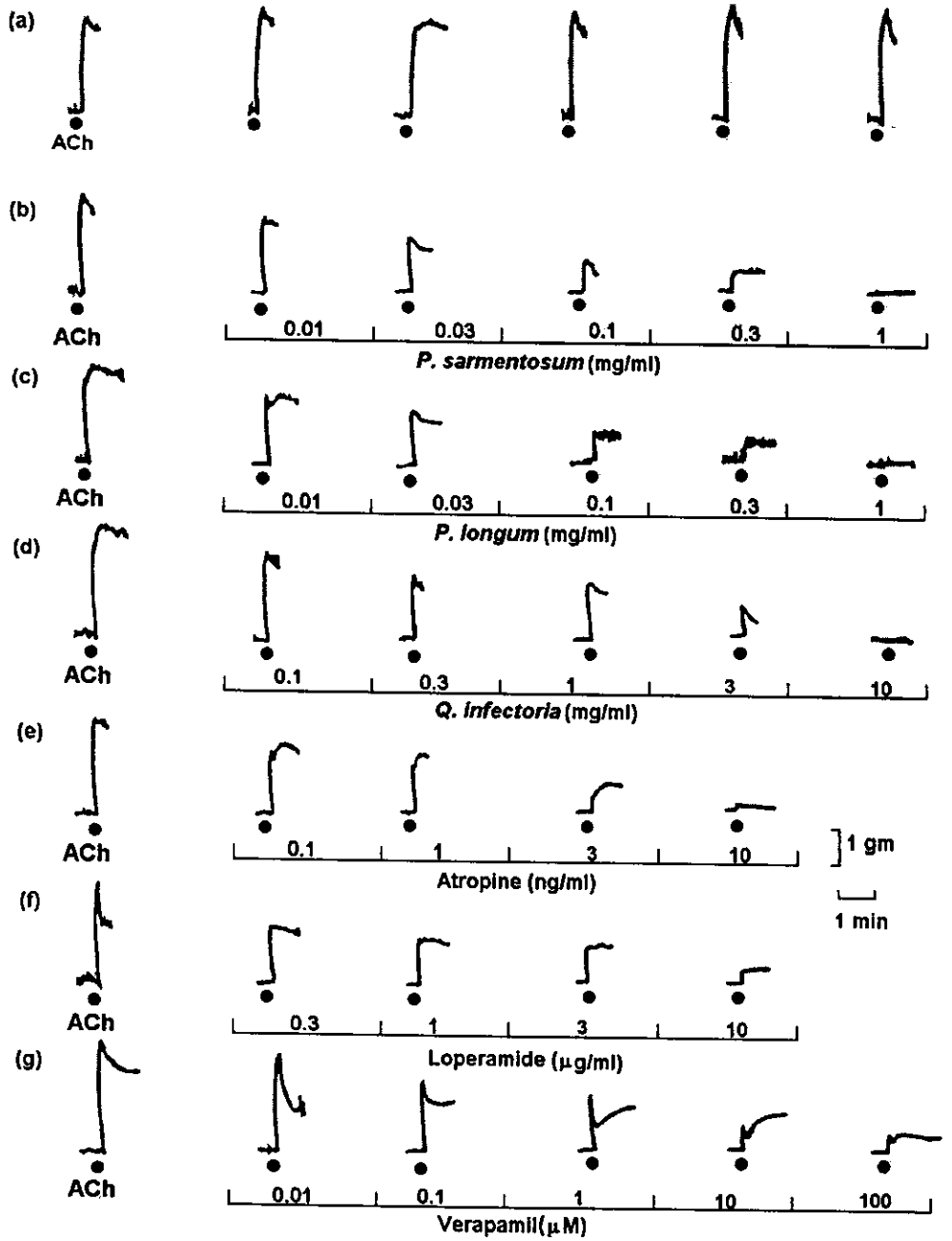


Figure 17 Representative recordings of the acetylcholine-induced contractions of isolated rat ileums and the inhibitions by various concentrations of the methanolic extracts of *P. sarmentosum* (b), *P. longum* (c) and Q. *infectoria* (d) in comparison with atropine (e), loperamide (f) and verapamil (g) and time control ileum (a).

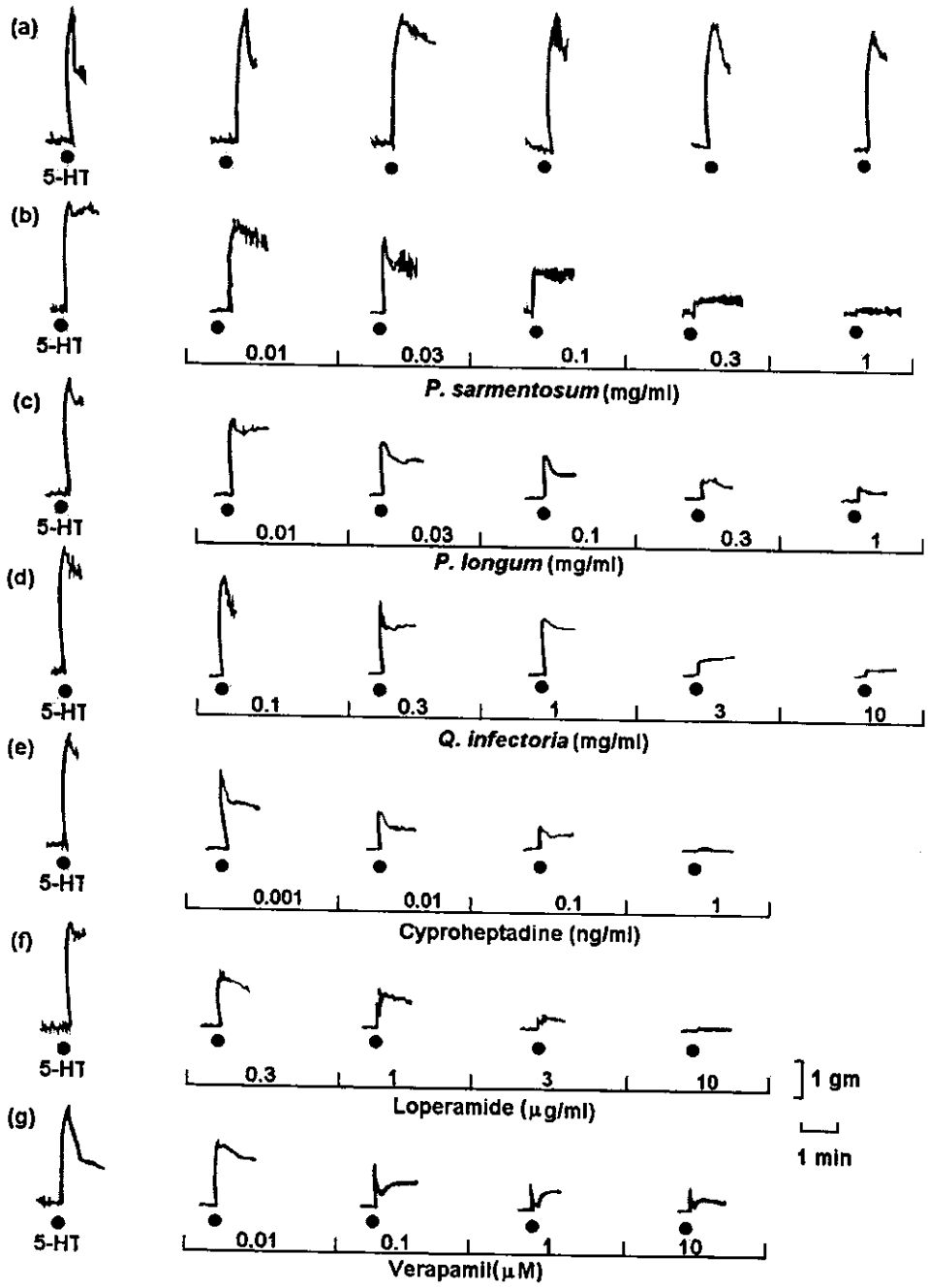


Figure 18 Representative recordings of the serotonin-induced contractions of isolated rat ileums and the inhibitions by various concentrations of the methanolic extracts of *P. sarmentosum* (b), *P. longum* (c) and *Q. infectoria* (d) in comparison with cyproheptadine (e), loperamide (f) and verapamil (g) and time control ileum (a).

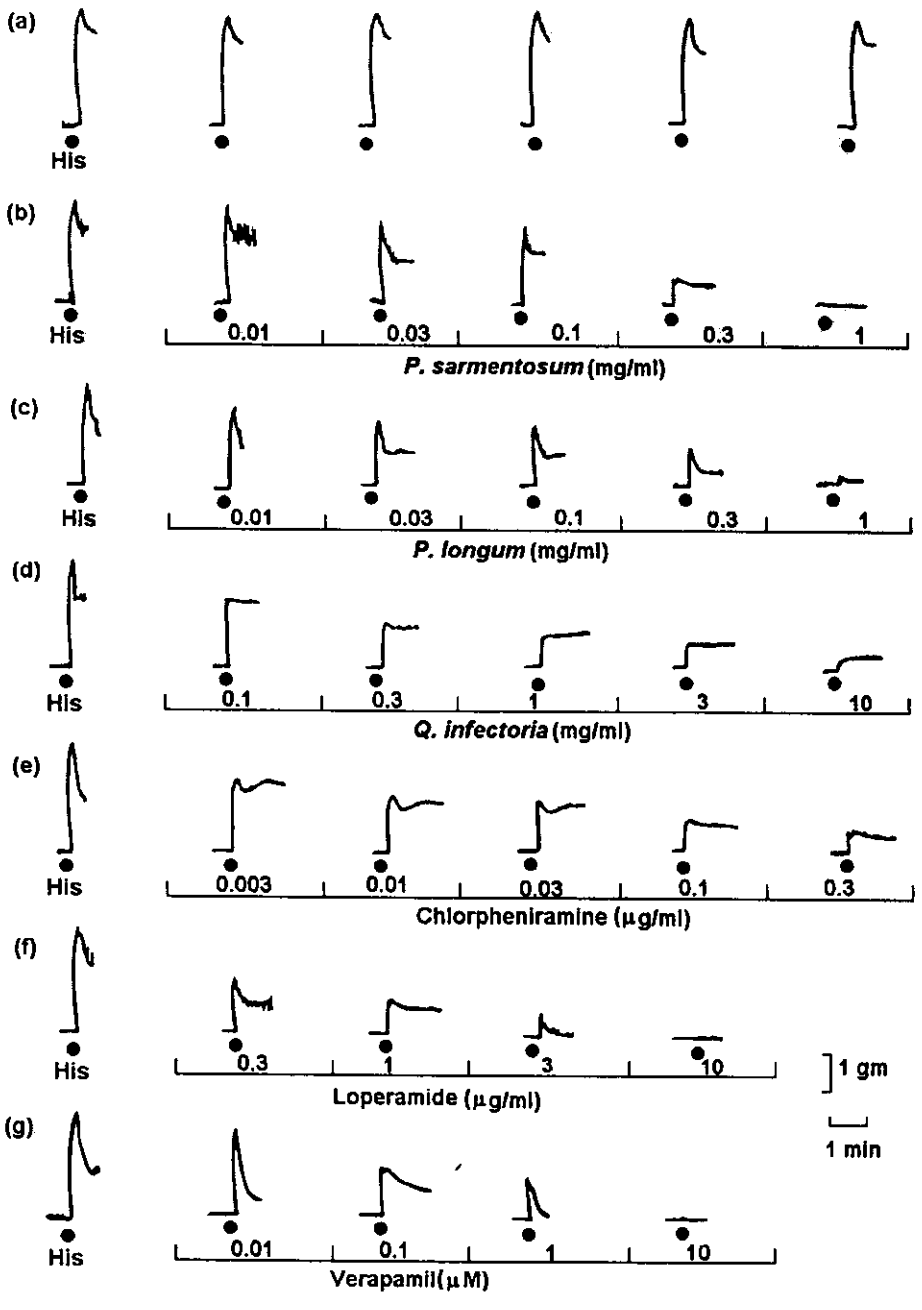


Figure 19 Representative recordings of the histamine-induced contractions of isolated guinea-pig ileums and the inhibitions by various concentrations of the methanolic extracts of *P. sarmentosum* (b), *P. longum* (c) and *Q. infectoria* (d) in comparison with chlorpheniramine (e), loperamide (f) and verapamil (g) and time control ileum (a).

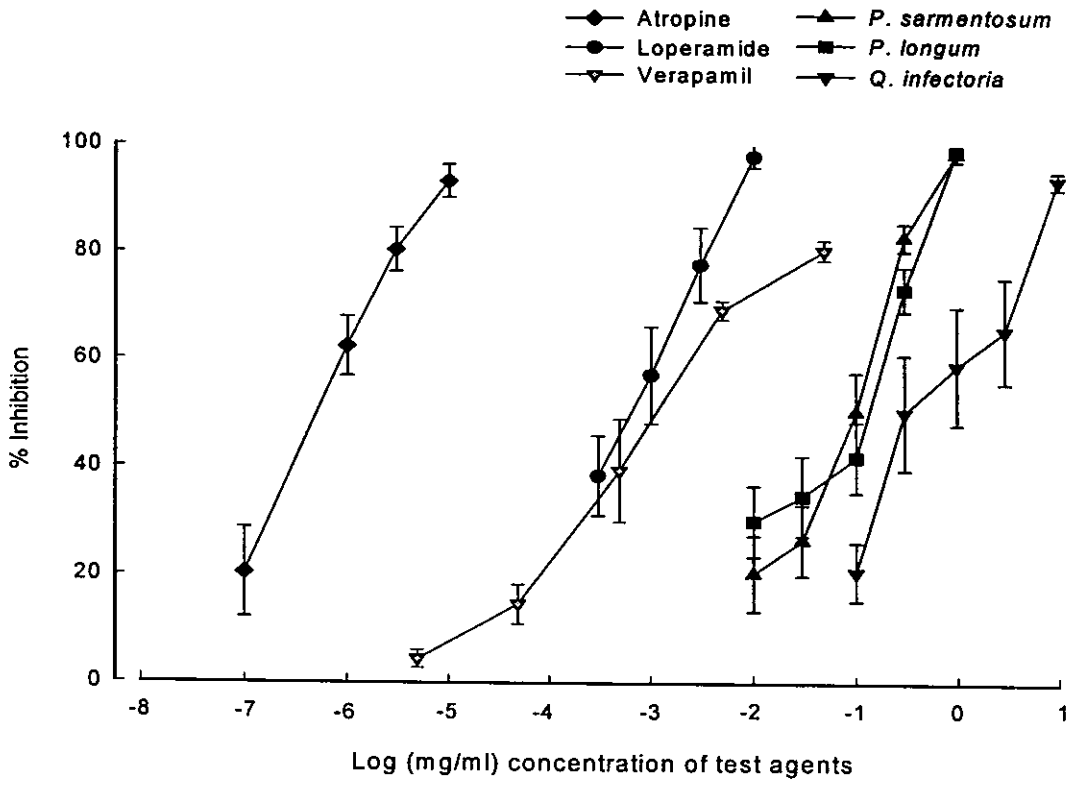


Figure 20 Inhibition of acetylcholine-induced contractions of isolated rat ileums by atropine (0.1 - 10 ng/ml), loperamide (0.3 - 10 mg/ml), verapamil (0.0049 - 49 mg/ml) and the methanolic extracts of *P. sarmentosum* (0.01 - 1 mg/ml), *P. longum* (0.01 - 1 mg/ml) and *Q. infectoria* (0.1 - 10 mg/ml). Vertical lines represent standard errors of means (n = 5).

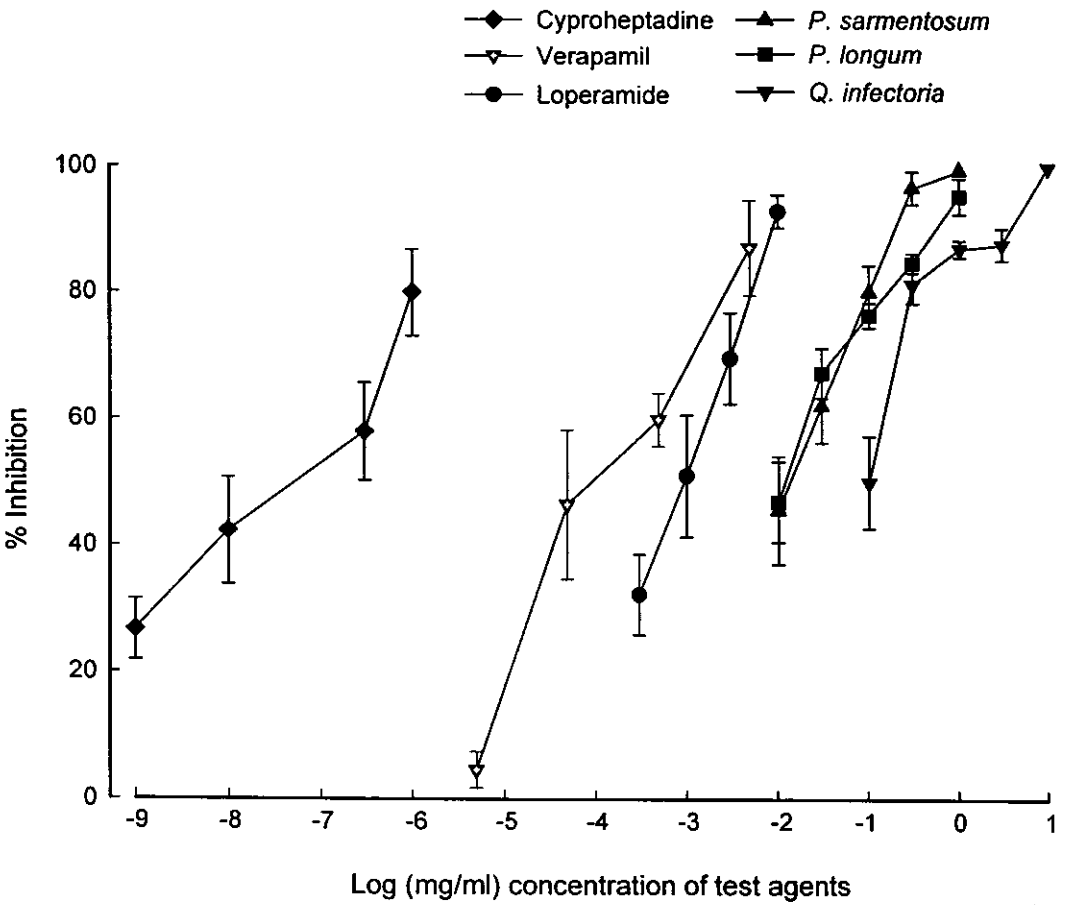


Figure 21 Inhibition of serotonin-induced contractions of isolated rat ileums by cyproheptadine (0.001 - 1 ng/ml), loperamide (0.3 - 10 mg/ml), verapamil (0.0049 - 4.9 mg/ml) and the methanolic extracts of *P. sarmentosum* (0.01 - 1 mg/ml), *P. longum* (0.01 - 1 mg/ml) and *Q. infectoria* (0.1 - 10 mg/ml). Vertical lines represent standard errors of means (n = 5).

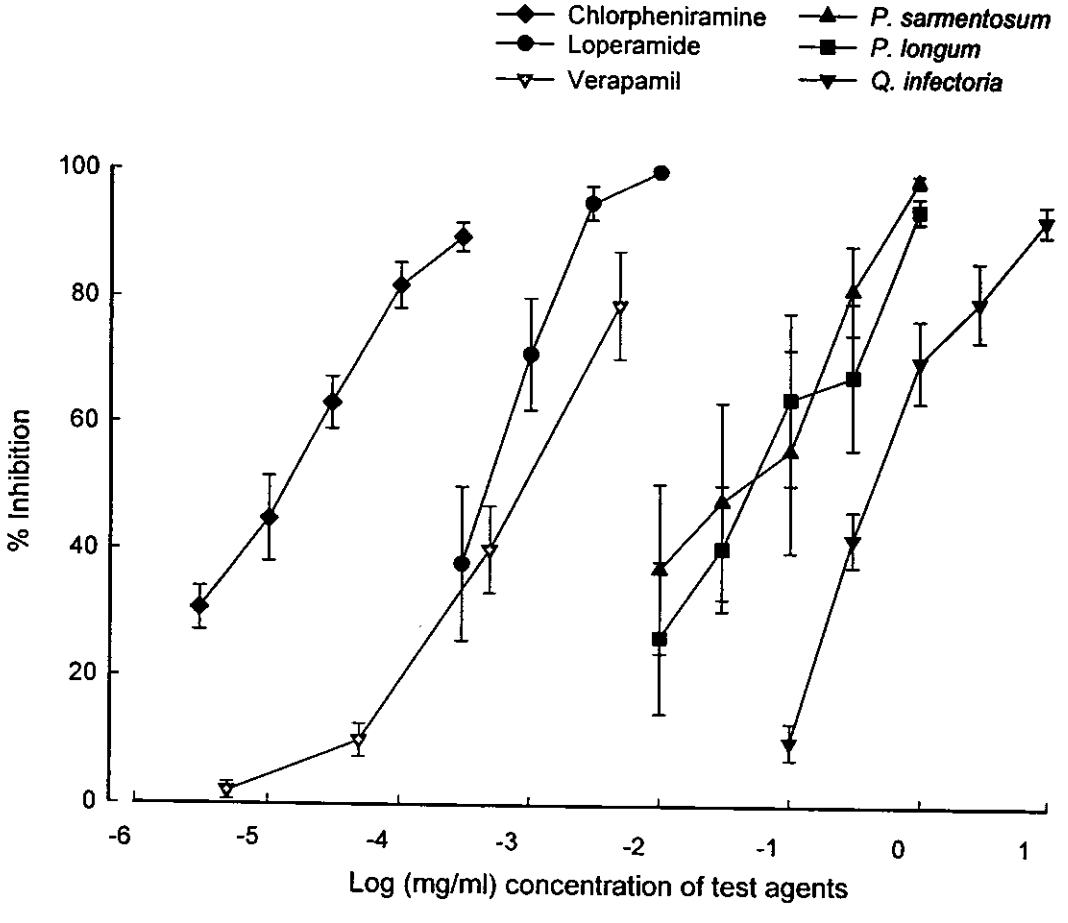


Figure 22 Inhibition of histamine-induced contractions of isolated guinea-pig ileums by chlorpheniramine (0.003 - 0.3 mg/ml), loperamide (0.3 - 10 mg/ml), verapamil (0.0049 - 4.9 mg/ml) and the methanolic extracts of *P. sarmentosum* (0.01 - 1 mg/ml), *P. longum* (0.01 – 1 mg/ml) and *Q. infectoria* (0.1 - 10 mg/ml). Vertical line represents standard errors of means (n = 5).

2.1.4 Effects of the receptor antagonists, atropine, chlorpheniramine and cyproheptadine

Atropine (0.1 - 10 ng/ml), chlorpheniramine (0.003 - 0.3 μg/ml) and cyproheptadine (0.001 - 10 ng/ml) significantly decreased the contractions induced by acetylcholine (10 μM), histamine (1 μM) and serotonin (1 μM), respectively. They behaved like loperamide and the plant extracts, reducing the spasmogen-induced contraction of ileums in the concentration-dependent fashion and completely abolished the contractions at their highest concentration used in this study (Figure 17 -19). Their concentration-response curves were also parallel to those of loperamide and the three plant-extracts (Figure 20 - 22). In addition, these receptor antagonists possessed the highest potency compared to loperamide, verapamil and the three plants extracts (Figure 20 - 22 and Table 5c)

2.2 Comparison of the effects of plant extracts and loperamide on the contractile responses of rat ileums to potassium chloride

High K^+ solution (30 mM) caused biphasic (phasic and tonic) contractions of rat ileum. Both components of the contractile responses were depressed by the three plants extracts and loperamide in the concentration-deepened manners (Figure 23, 24 and Table 6). Table 7 compares the potencies of the test agents in terms of IC_{50} and relative potency ratios similar to the inhibitory effects on the agonist-induced contractions as mentioned above, the order of potency was *P. sarmentosum* = *P. longum* > *Q. infectoria.* The IC_{50} values calculated for *P. sarmentosum* and *P. longum* on both phases of KCl-induced contractions did not differ significantly ($p>0.05$) from one another, but each of them was significantly different ($p<0.05$) from that of *Q. infectoria.* In comparison of their suppression on the phasic contraction to tonic contraction, there were no significant differences obtained for all plant extracts or loperamide (Table 7).

Table 4 IC_{50} values (concentration producing 50% of maximum inhibition of agonist-induced contractions of rat or guinea-pig isolated ileums, n= 5) of the three plant methanolic extracts, loperamide, verapamil, the muscarinic receptor antagonist, atropine; the serotoninergic receptor antagonist, cyproheptadine and the histaminergic receptor antagonist, chlorpheniramine.

Compounds	IC_{50} (95% confidence limit) µg/ml		
	Agonists		
	Acetylcholine (10 µM)	Serotonin (3 µM)	Histamine (1 µM)
P. sarmentosum	88 (66-117)	13 (7-23)	44 (14-135)
P. longum	91 (65-130)	6 (2-13)	54 (20-145)
Q. infectoria	343 (95-1239)	37 (10-131)	377 (203-701)
Loperamide	0.61 (0.37-1.02)	0.89 (0.57-1.39)	0.42 (0.20-0.82)
Verapamil	0.44 (0.16-1.26)	0.89 (0.57-1.39)	0.42 (0.20-0.82)
Atropine	$5.4\ (3.6\text{-}8.1)\times10^{-4}$	-	-
Cyproheptadine	-	$2.6\ (1.1\text{-}6.4)\times10^{-5}$	
Chlorpheniramine	-		$7.5\ (1.3\text{-}4.3)\times10^{-4}$

Table 5 Comparison of the potencies (on μg/ml concentration basis) of the methanolic extracts of *P. sarmentosum*, *P. longum* and *Q. infectoria* on the inhibition of the agonist-induced contractions of rat or guinea-pig ileums with those of: (a) loperamide (Lop) and (b) verapamil (Ver) and (c) atropine (Atrop), cyproheptadine (Cypro) and chlorpheniramine (CPM) (n=5).

(a)

Lop:extract	Potency ratios (in inhibition of contraction induced by various agonists)		
	Acetylcholine (10 μM)	Serotonin (3 μM)	Histamine (1 μM)
Lop : *P. sarmentosum*	1: 129	1: 15	1: 139
Lop : *P. longum*	1: 133	1: 18	1: 187
Lop : *Q. infectoria*	1: 1,023	1: 96	1: 1,380

(b)

Ver:extract	Potency ratios (in inhibition of contraction induced by various agonists)		
	Acetylcholine (10 μM)	Serotonin (3 μM)	Histamine (1 μM)
Ver : *P. sarmentosum*	1: 46	1: 12	1: 51
Ver : *P. longum*	1: 52	1: 28	1: 72
Ver : *Q. infectoria*	1: 337	1: 67	1: 501

(c)

Antagonist : extract	Potency ratios (in inhibition of contraction induced by various agonists)		
	Acetylcholine (10 μM)	Serotonin (3 μM)	Histamine (1 μM)
Atrop : *P. sarmentosum*	1: 1.29×10^5	-	-
Atrop : *P. longum*	1: 1.45×10^5	-	-
Atrop : *Q. infectoria*	1: 1.10×10^6	-	-
Cypro : *P. sarmentosum*	-	1: 2.51×10^5	-
Cypro : *P. longum*	-	1: 3.51×10^5	-
Cypro : *Q. infectoria*	-	1: 1.47×10^6	-
CPM : *P. sarmentosum*	-	-	1: 2.91×10^3
CPM : *P. longum*	-	-	1: 4.00×10^3
CPM : *Q. infectoria*	-	-	1: 2.88×10^4

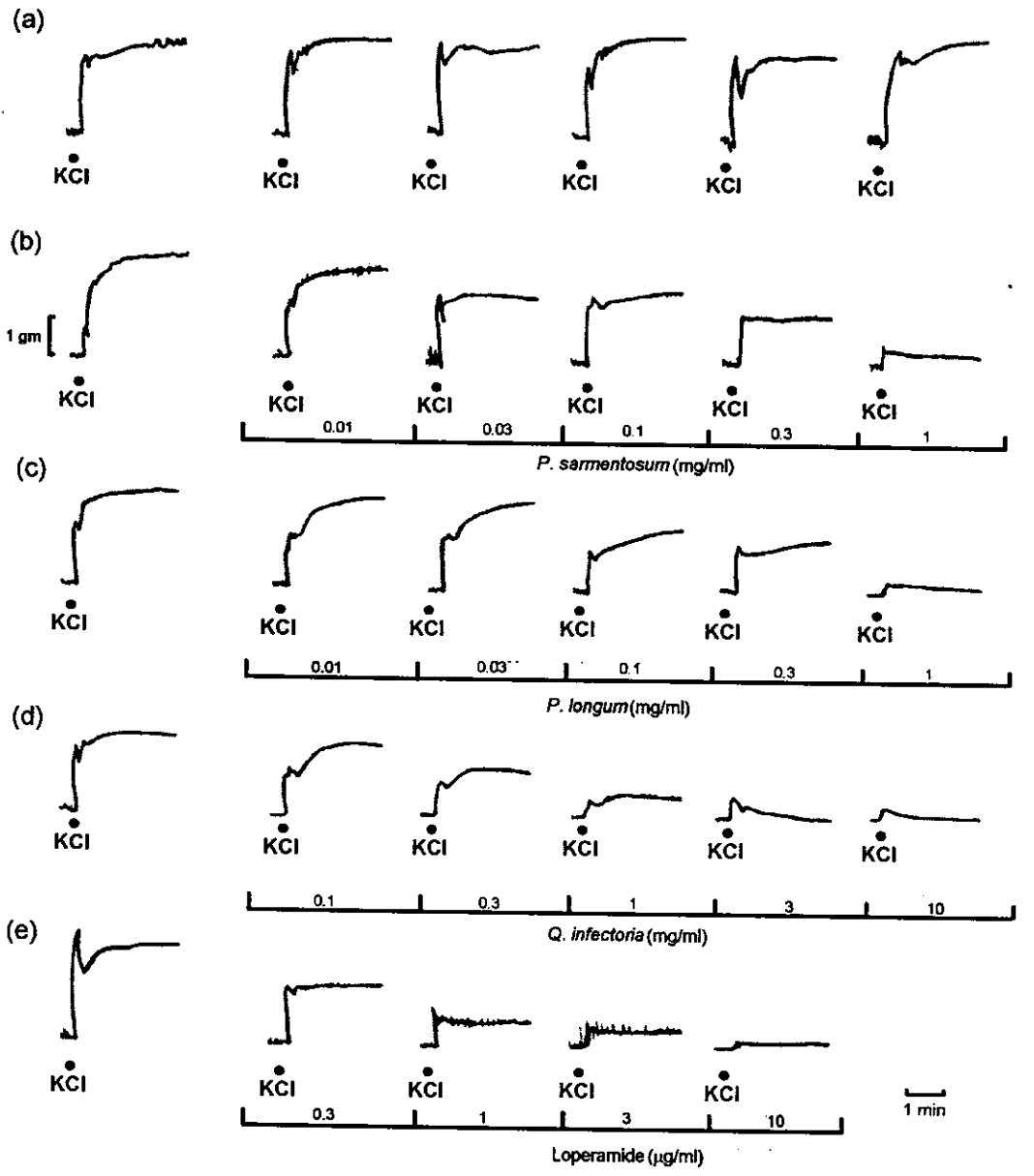


Figure 23 Representative recordings of potassium chloride-induced contractions (each consists of phasic, initial rapid contraction, followed by a sustained tonic contraction) of rat ileums and the inhibition by various concentrations of the methanolic extracts of *P. sarmentosum* (b), *P. longum* (c) and *Q. infectoria* (d) in comparison with loperamide (e) and time control ileum (a).

Table 6 The inhibitory effects of *P. sarmentosum*, *P. longum* and *Q. infectoria* methanolic extracts and loperamide on the phasic and tonic contraction-induced by KCl of rat ileums (data are expressed as mean ± SE, n=5)

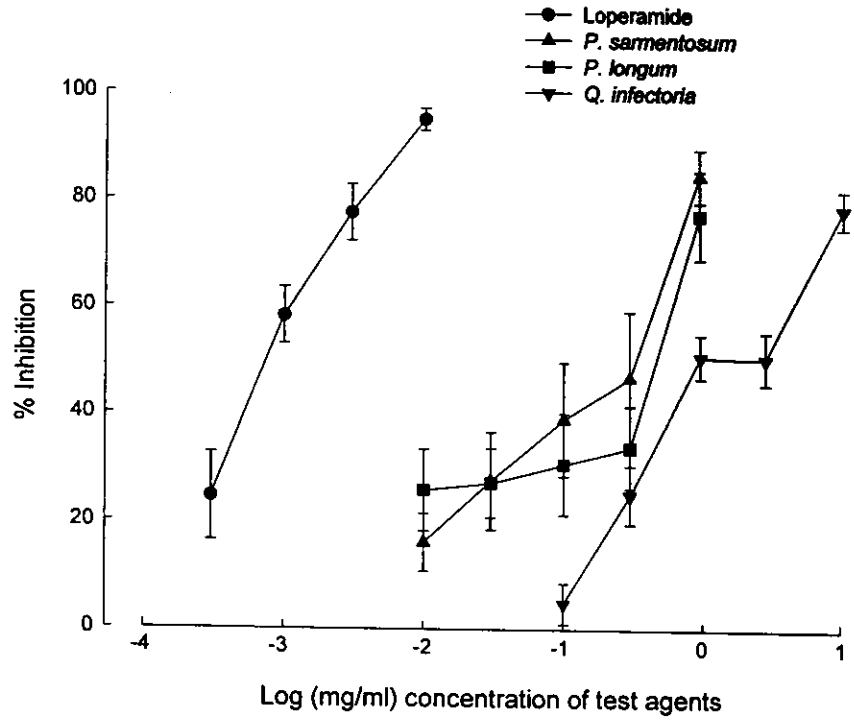
Compounds		% inhibition of contraction-induced by KCl (30 mM)	
		Phasic contraction	Tonic contraction
P. sarmentosum	0.01 mg/ml	16.13 ± 5.44*	17.41 ± 10.69
	0.03 mg/ml	27.61 ± 9.02*	28.01 ± 17.61
	0.1 mg/ml	39.09 ±10.58*	42.72 ± 19.59
	0.3 mg/ml	46.82 ±12.44*	47.67 ± 20.89
	1.0 mg/ml	84.68 ± 4.97*	87.59 ± 5.92*
P. longum	0.01 mg/ml	25.90 ± 7.61*	1.97 ± 1.55
	0.03 mg/ml	27.21 ± 1.44*	1.67 ± 1.67
	0.1 mg/ml	30.74 ± 9.41*	6.95 ± 5.01
	0.3 mg/ml	33.91 ± 7.68*	27.44 ± 8.59*
	1.0 mg/ml	77.38 ± 8.24*	82.59 ± 8.20*
Q. infectoria	0.1 mg/ml	4.74 ± 3.78	9.00 ± 3.92
	0.3 mg/ml	25.04 ± 5.43*	30.35 ± 6.80*
	1.0 mg/ml	50.91 ± 4.10*	55.37 ± 5.88*
	3.0 mg/ml	50.56 ± 4.87*	70.85 ± 4.02*
	10.0 mg/ml	78.64 ± 3.48*	94.44 ± 2.66*
Loperamide	0.3 µg/ml	24.63 ± 8.19*	22.30 ± 4.89*
	1.0 µg/ml	58.36 ± 5.43*	54.48 ± 4.41*
	3.0 µg/ml	77.68 ± 5.18*	78.86 ± 3.95*
	10.0 µg/ml	78.64 ± 3.48*	95.06 ± 3.74*

* Significant differences from the initial contraction

Table 7 The IC_{50} values of the three plant extracts and loperamide on the inhibition of phasic and tonic contractions of KCl-induced contraction of rat ileums and their potency ratios (on (µg/ml concentration basis) on the two phases of contractions (n=5).

Compounds	IC50 (confidence limit) (µg/ml)		ratio phasic/tonic contraction	Potency ratio Lop : extract	
	Phasic contraction	Tonic contraction		Phasic	Tonic
P. sarmentosum	153 (79-299)	157 (53-463)	0.97	1 : 206	1 : 166
P. longum	264 (123-563)	406 (253-651)	0.65	1 : 321	1 : 520
Q. infectoria	932 (609-1426)	848 (612-1175)	1.09	1 : 1849	1 : 926
Loperamide	0.86 (0.61-1.20)	0.88 (0.66-1.18)	0.97		

(A)



(B)

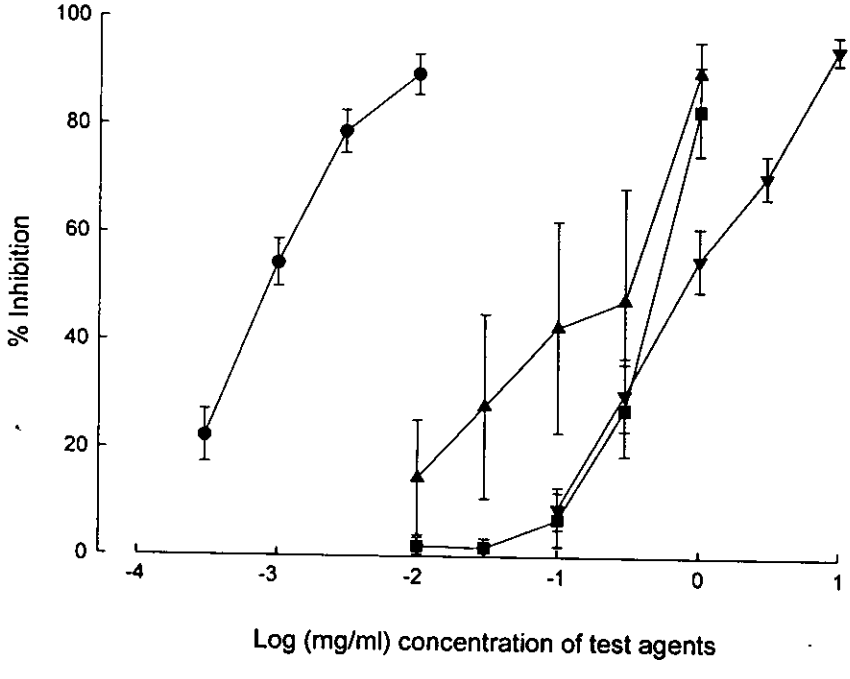


Figure 24 Inhibition of potassium chloride-induced phasic contraction (A) and tonic contraction (B) of rat ileums by loperamide (0.3-10 μg/ml), *P. sarmentosum* (0.01-1 mg/ml), *P. longum* (0.01-1 mg/ml) and *Q. infectoria* (0.1-10 mg/ml).

2.3 Comparison of the effects of plants extracts, loperamide and verapamil on the contractile responses of guinea-pig ileums to calcium chloride

In guinea-pig ileal preparations contracted with calcium chloride, *P. longum* (0.1 - 1 mg/ml), *P. sarmentosum* (0.1 - 1 mg/ml), *Q. Infectoria* (1 - 10 mg/ml), the reference drug, loperamide (0.1 - 1 μg/ml) and the calcium channel antagonist, verapamil (10^{-8} - 10^{-7}M or 4.9 - 49 ng/ml), inhibited significant rightward shift of the concentration response curves of calcium chloride in a concentration dependent manner; except the concentration-response line of 0.3 mg/ml *P. sarmentosum* that was not significantly different from control. Typical traces were shown in Figure 25. Data were summarized in Figure 26 - 28.

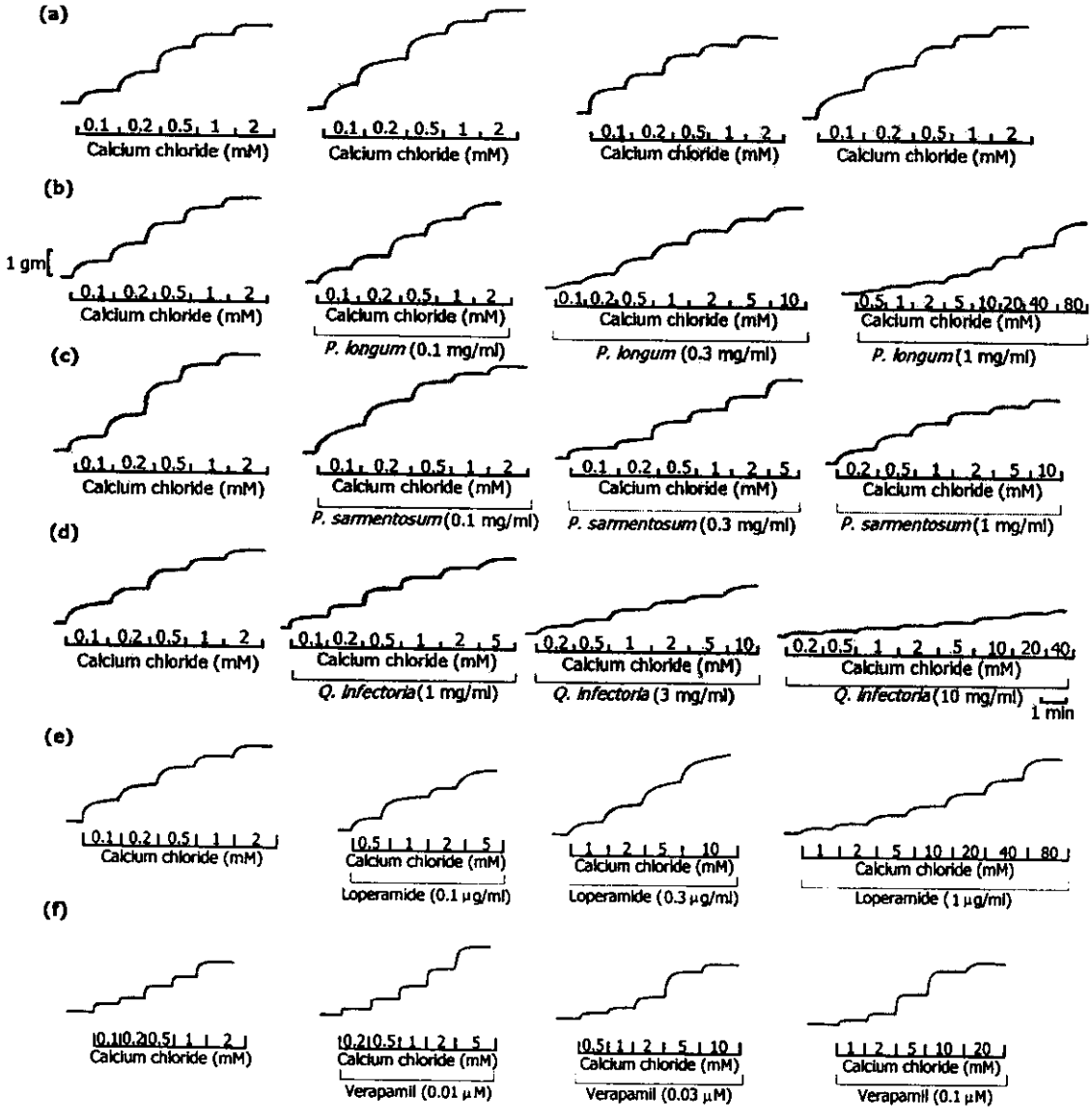


Figure 25 Representative recordings of the effects of the methanolic extract of (b) *P. longum* (0.1-1 mg/ml), (c) *P. sarmentosum* (0.1-1 mg/ml), (d) *Q. infectoria* (1-10 mg/ml), (e) loperamide (0.1-1 µg/ml) and (f) verapamil (0.01-0.1 µM) on the contractions of guinea pig ileum-induced by calcium chloride (added cumulatively), in comparison with those of time control (a).

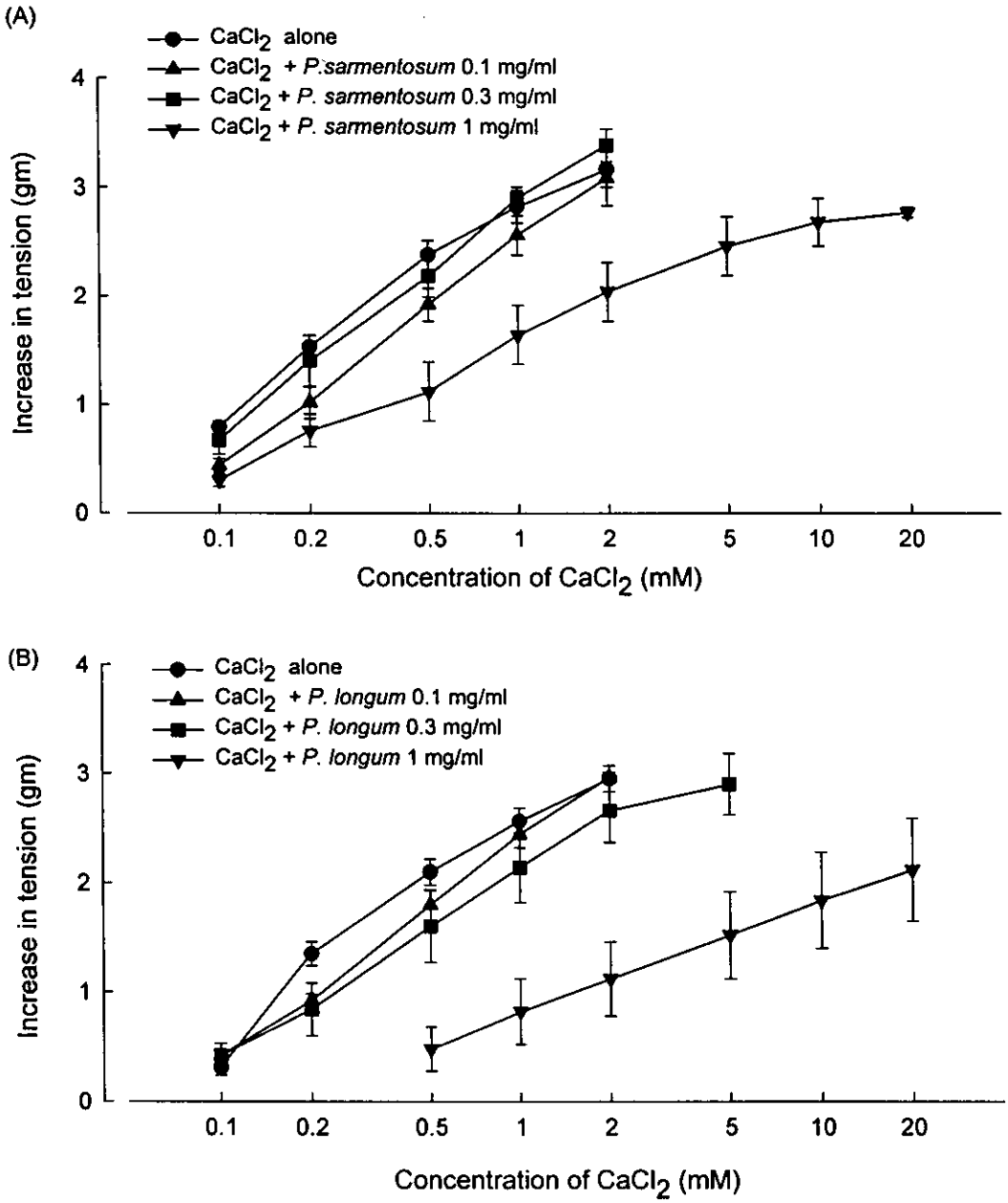


Figure 26 Cumulative concentration-effects of calcium chloride induced-contractions of isolated guinea-pig ileums and the antagonisms by (A) *P. sarmentosum* methanolic extract (0.1-1 mg/ml) and (B) *P. longum* methanolic extract (0.1-1 mg/ml). Vertical lines represent standard errors of means (n=5).

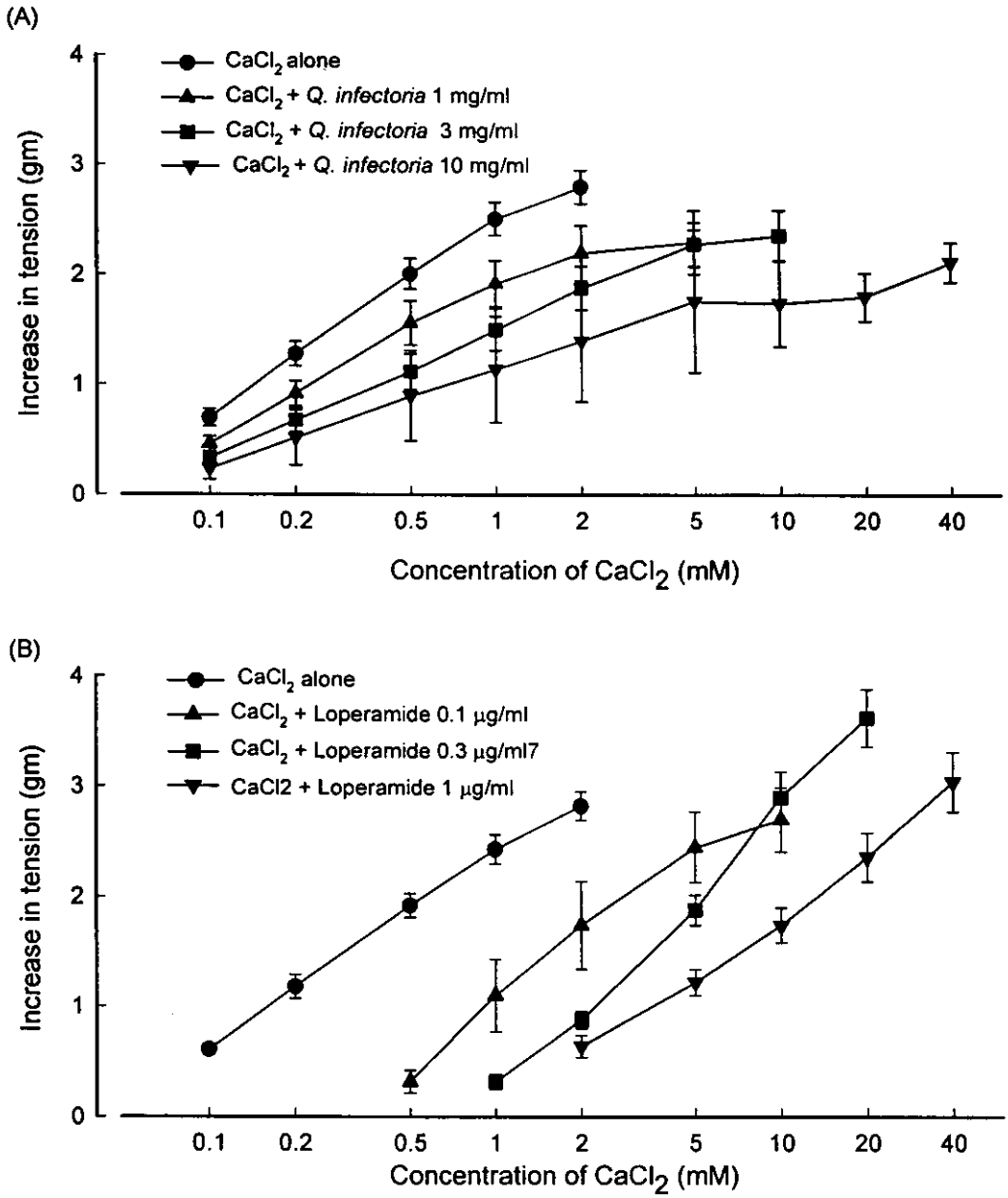


Figure 27 Cumulative concentration-effects of calcium chloride induced-contractions of isolated guinea-pig ileum and the antagonisms by (A) *Q. infectoria* methanolic extract (1-10 mg/ml) and (B) loperamide (0.3-3 mg/ml). Vertical lines represent standard errors of means (n=5), which are smaller than the size of symbols in some cases.

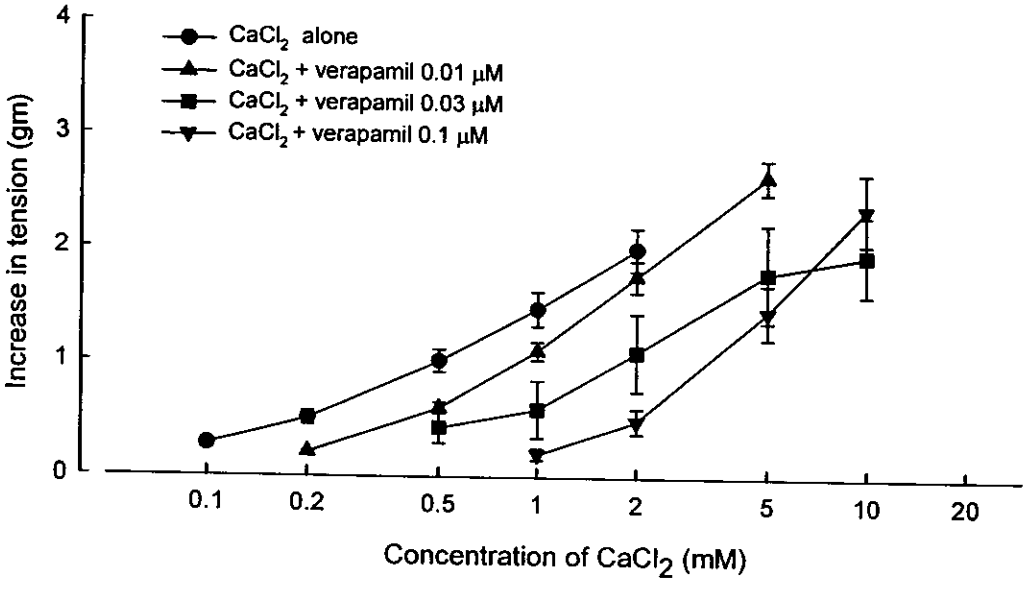


Figure 28 Cumulative concentration-effects of calcium chloride-induced contraction of isolated guinea-pig ileums and the antagonisms by verapamil (0.01-0.1 mM or 0.0049-0.049 mg/ml). Vertical lines represent standard errors of means (n=5), which are smaller than the size of symbols in some cases.