Chapter II

Materials and Methods

1. Experimental Animals.

The Albino rats of wistar strain, guinea-pigs and mice of male sex were used in this investigation. The rats weighing 250-300 g, guinea-pig weighing 300-400 g, and mice weighing 25-40 g . The rats and mice were supplied by the Animal center, Faculty of Science, Mahidol University. They were fed with standard rat chow obtained commercially and tap water ad libitum.

2. Preparation of The Crude extracts.

A fresh leaves of Cymbopogon citratus were used in this investigation and then washed and dried. The concentration of crude extract from C. citratus were used generally are ratio of weight in gram of the fresh leaves of C. citratus and volume of water in milliter such as 1:1, 2:1, 3:1, 4:1, and 5:1. The concentration 1:1 of crude extract was preparated by 40 g of the fresh leaves in 100 ml of hot water for 10 min . The crude extract was filtered and evaporated in hot air oven at 60^{o}-70^{o} C, to maintain the crude extract only 40 ml (1 ml of the final solution was equivalent to 1 g of the fresh leaves of C. citratus; 1:1). The concentration 1:1 of crude extract was diluted by water in order to have the concentrations of crude extract

of 0.0625, 0.125, 0.25 and 0.50 respectively. The concentration of 2:1, 3:1, 4:1 and 5:1 of the crude extract were similarly obtained with the concentration 1:1 preparation, by changing mass of the fresh leaves of C. citratus and/or volume of water.

The concentration 2:1 was preparated by 50 g of the fresh leaves in 100 ml of water extract for 10 min. The extract was filtered and evaporated in hot air oven at 60^{o}-70^{o} C, to maintain the final solution to 25 ml, and 60 g of the fresh leaves in 100 ml of hot water were extracted for 10 min and decreased volume in order to maintain the final solution to 20 ml for the concentration 3:1.

The concentration 4:1 and 5:1 were preparated by 40 g and 50 g of the fresh leaves and 100 ml of hot water were extracted for 10 min, filtered and evaporated in hot air oven, to maintain the final solution to 10 ml, respectively.

All concentration of crude extract were determined for cations concentrations (Na^{+} , K^{+} and Ca^{2+}) by Atomic Absorbtion / Flame Emission Spectrophotometer (Model AA-630-12, Shimadzu.) and determined for pH by pH meter. (Model. CG. 819, Schott Gerate.) These crude extract were stored in the refrigerator for no longer than one week.

3. Preparation of The Isolated Rat Stomach Fundus Strip.

The rat was killed by a stunning on the head and cutting the throat. After that the abdomen was opened, the fundal part of the

stomach could easily be identified because it is grey whereas the pyloric part is pink. It was cut away, opened out longitudinally, placed in a dish containing Tyrode's solution of the following composition (in g/l) NaCl 8.0 g, KCl 0.2 g, $MgCl_2$ 0.1 g, $CaCl_2$ 0.2 g, NaH_2PO_4 0.05 g, $NaHCO_3$ 1.0 g and glucose 1.0g with the temperature controlled at 37^0 C aerated with mixture of oxygen (O_2) 95% and carbondioxide (CO_2) 5% . The tissue was made into a strip about 1-2 cm long by suitable transverse cuts. The strip of stomach was suspended in a 30 ml organ bath containing Tyrode's solution at 37^o C aerated with 95% O_2 + 5% CO_2.

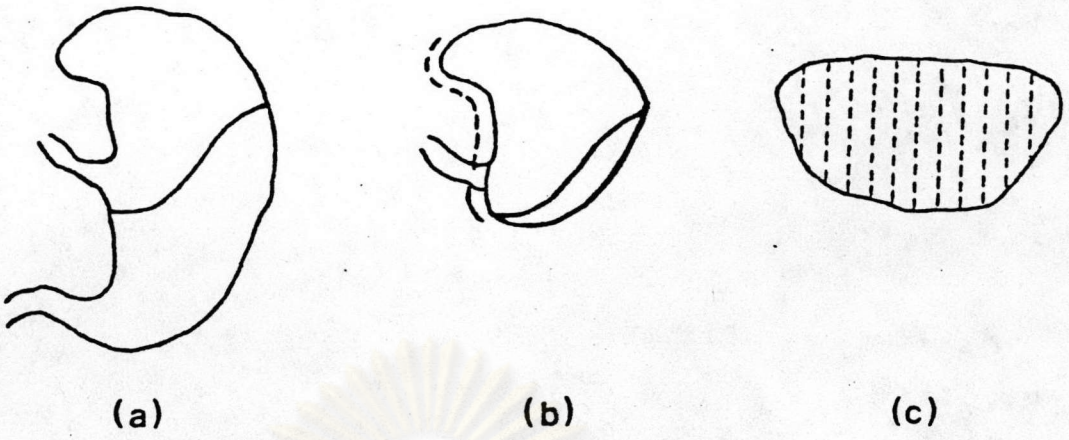
4. Preparation of The Isolated Rat Duodenum Seqment.

Experiment was carried out on 6-8 cm segment of duodenum from rat of male sex. The rat was killed by stunning blow on the head. The abdomen wall was opened, the duodenum was excised and 6-8 cm segment and were removed from the next 1 cm of stomach. Their contents were gently removed by washing with Tyrode's solution.

5. Preparation of The Isolated Guinea-pig Ileum Segment.

The guinea-pig was killed by a stunning. The abdomen wall was opened, the ileum was excised and 6-8 cm each segment was removed from the next 10 cm of ileocaecal junction. Their contents were gently removed by washing with Tyrode's solution.

A.



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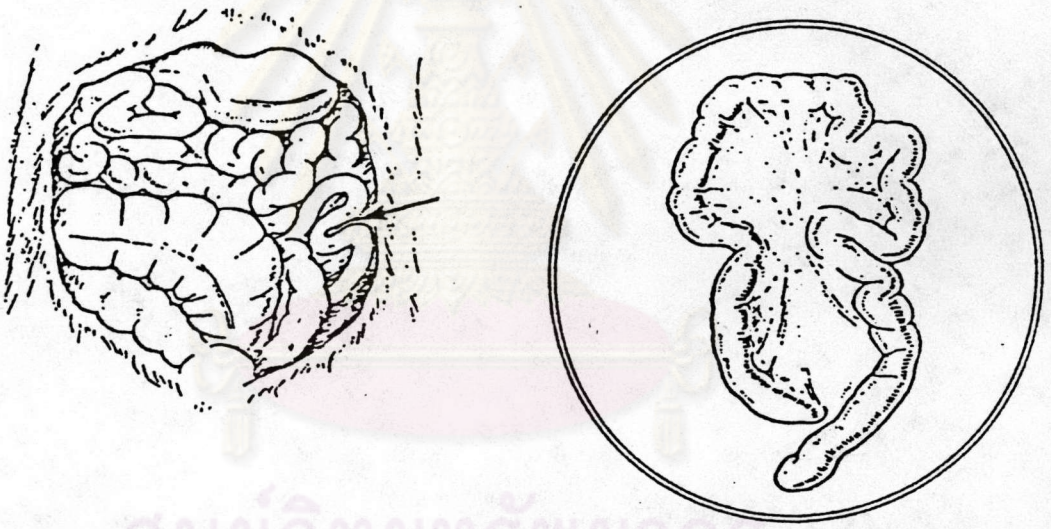

Fig 2 The isolated organ preparation :

A. Isolated rat stomach fundus strip; (a), the whole stomach; (b) the fundus part and then cut along the dotted line; (c), the fundus opened and cut strips.

B. Isolated quinea-pig ilieum segment.

6. Preparation of The Isolated Mouse Stomach.

The mouse was killed by a sharp blow on the head and the abdomen was opened, the stomach was excised, placed in a dish containing Tyrode's solution and their contents were gently removed by washing with Tyrode's solution.

All the isolated tissues in this studies were suspended in a 30 ml organ bath containing Tyrode's solution at 37^{o} C aerated with 95% O_2 + 5% CO_2. One end of the tissue was attached to a hook of the glass rod in the organ bath while the other end attached to a force-displacement transducer FT 03 C which to Polygraph recorder (Grass Model 5D). The tissues were equilibrated for 60 minutes under a resting tension of 1 g before being exposed to any drug. During the equilibration period, the Tyrode's solution was changed every 30 min. Isometric contraction were recorded with Polygraph recorder.

7. Coaxial Electrical Stimulation on The Isolated Guinea-pig Ileum Segment.

An ileum segment, about 2-3 cm long was threaded on a platinum electrode with attachment of one end to a bottom support and the other end to a force-displacement transducer FT 03 C which to Polygraph recorder (Grass Model 5D.). The electrode was situated within the lumen. A second platinum electrode was dipping into the oxygenated (95% O_2 + 5% CO_2) Tyrode's solution made the whole bath a diffuse external electrode (Fig 3). The preparation was excited with stimuli

of 3,5 and 10 volts at a frequency of six per second, with a square wave pluse duration of 0.5 msec. Stimuli were elicited by a Grass SD 9. Stimulation . The tension developed and amplitude during muscle contraction were recorded by an isometric force-displacement transducer FT 03 C and Polygraph recorder (Grass Model 5D.).

8. Organ Bath and Heater Controller.

The organ bath used in the isolated preparation of this studies, in bath of heater controller (BioScience, 61310-1) was of single wall type. This organ bath containing 30 ml of Tyrode's solution and had a glass rod for attached tissue preparation, and there was the other glass rod for through a mixture gas for tissue. The temperature of Tyrode's solution within organ bath was controlled at 37^{o} C by water temperature between the bath and organ bath of the heater controller and a circulating water pump of Ultrathermostat (Heto denmark). (Fig 4)

9. Drugs and Chemicals.

Drugs : The crude extract from Cymbopogon citratus. , devided into 9 doses :

dose 1. at concentration	0.0625	g/ml
dose 2. at concentration	0.125	g/ml
dose 3. at concentration	0.25	g/ml
dose 4. at concentration	0.50	g/ml

dose 5.	at concentration	1.00	g/ml	(1:1)
dose 6.	at concentration	2.00	g/ml	(2:1)
dose 7.	at concentration	3.00	g/ml	(3:1)
dose 8.	at concentration	4.00	g/ml	(4:1)
dose 9.	at concentration	5.00	g/ml	(5:1)

Chemicals. :

Acetylcholine chloride (Sigma)

Histamine dihydrochloride (Sigma)

5-Hydroxytryptamine creatinine sulphate (Sigma)

Atropine sulphate (Sigma)

Chlorpheniramine (The Government Pharmaceutical Organization)

Cimetidine (The Government Pharmaceutical Organization)

Cyproheptadine hydrochloride (Sigma)

Absolute ethanol (Merck)

NaCl, KCl and $MgCl_2$ (May and Baker)

$CaCl_2$ (BDH)

$NaHCO_3$ (Riedel-de Haoeon)

NaH_2PO_4 and Glucose (Merck)

Most chemicals were dissolved in deionized - distilled water, except cyproheptadine hydrochloride was dissolved in 70% ethanol.

10. Drugs and Chemicals Administration.

After the isolated tissues have been equilibrated for 60 min

in the organ bath, the drugs and chemicals were administered into the bath fluid in a cumulative regimen using a syringe (1.00 cc). Additionally, the drug was administrated into the bath fluid in a non-cumulative regimen by washed after 5 min for the addition of each dose of the drug. The volume of drug used 0.2 and 0.3 ml, chemicals used 0.1 ml. The volume of drugs and chemicals used did not cause any significant change in the ionic concentration of the physiological salt solution.

11. Data Acquisition.

In each experiment a dose-response curve was constructed, usually employing five to ten doses. The concentration of the agonist and antagonists were expressed in molar (M) of base in final bath concentration.

Results were expressed as means and standard deviation (S.D.) of the means. Significance of the differences between control and drug-treated means were determined using "Student's t-test.". Values of P. is less than 0.05 were taken to implicate statistical significance.

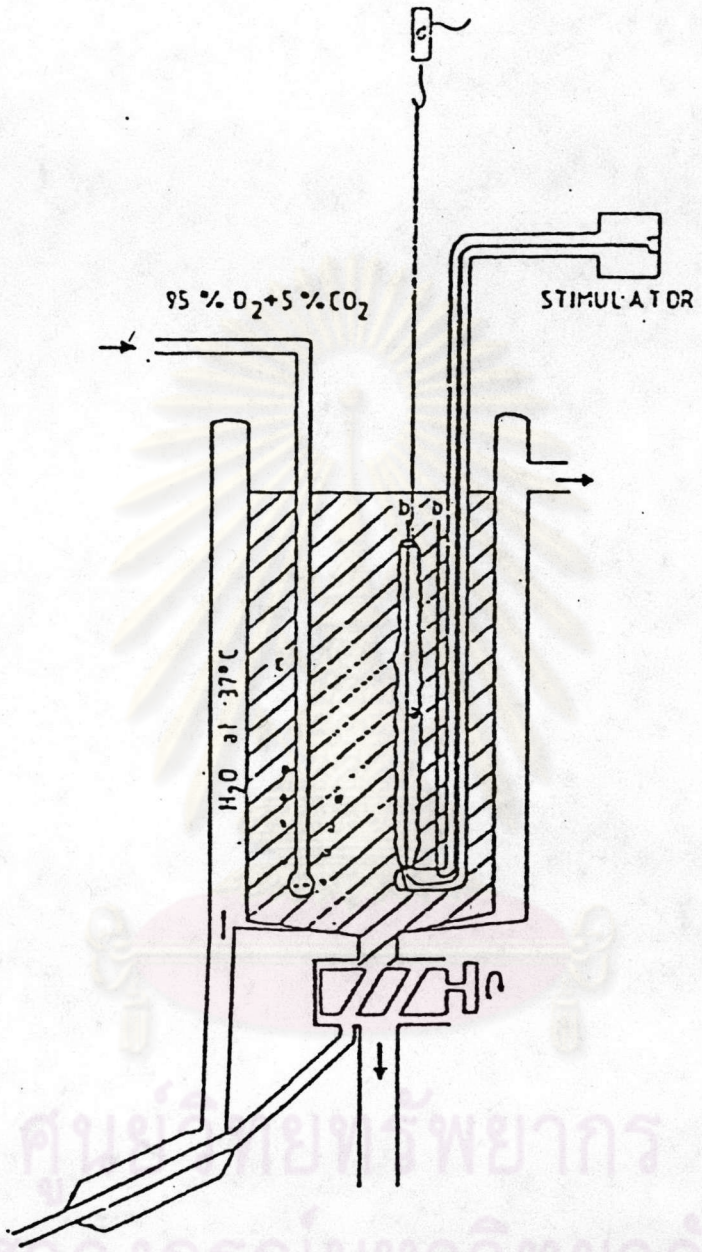

Fig 3 Coaxial electrical stimulation. Experimental set- up. a: quinea-pig ilieum segment; b: platinum electrode and c: glass rod with a 95% O_2 and 5% CO_2 mixture.

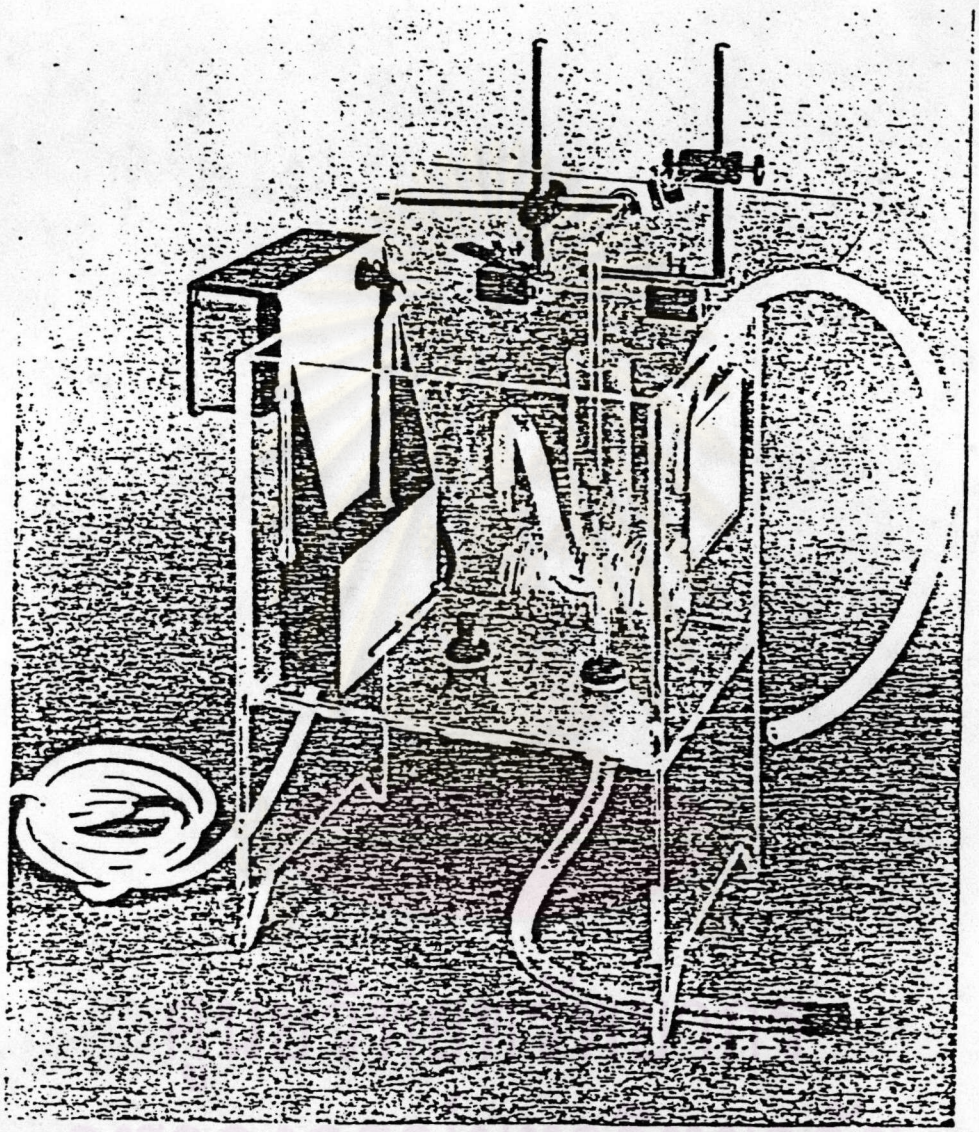

Fig 4 Organ Bath and Heater controller (BioScience).

Table 1 The compositions of chemicals and electrolytes of Tyrode's solution.

Chemicals	g/l	Electrolytes	mEq/l
NaCl	8.0	Na^+	149.2
KCl	0.2	K^+	2.7
$MgCl_2$	0.1	Mg^{2+}	2.1
$CaCl_2$	0.2	Ca^{2+}	3.6
NaH_2PO_4	0.05	$H_2PO_4^-$	0.4
$NaHCO_3$	1.0	HCO_3^-	11.9
Glucose	1.0	--	5.0
		Cl^-	145.3

Aerating pass : 95% O_2 + 5% CO_2

pH : 7.4