CHAPTER 3

DESIGN OF INVESTIGATION
The present investigation aims at exploring:

(a) some pharmacodynamic aspects of crude *Heterometrus bengalensis* venom, i.e., apart from those reported by Heg Chowdhury (1976) from this laboratory,

(b) studies on a hitherto-unknown smooth muscle contractile substance (a) which was found to occur in the venom of *Heterometrus bengalensis*, including attempts at its separation, characterization and pharmacodynamics,

(c) isolation and characterization of the phospholipase activity detected in venom.

In this context, the following design of investigation was adopted.

I. Experiments concerning pharmacodynamics of crude venom and its enzyme constituents.

1. Pharmacodynamic actions

Effect on blood pressure

- in guinea-pig
- in rat

1.2 Effect on capillary permeability (dye extravasation)

1.3 Mast cell degranulation.
2. Enzyme constituents
2.1 Phospholipase
2.2 Cholinesterase

II. Experiments concerning the findings of a smooth muscle contractile substance (s) and attempt at its separation, characterisation and pharmacodynamics.

1. Attempts at separation
1.1 Single solvent extraction procedure
1.1.1 Ethanol (95%)
1.1.2 Methanol
1.1.3 Butanol
1.1.4 Ether
1.1.5 Ethyl acetate
1.1.6 Acetone

With each solvent extraction the biological activity of supernatant and precipitate were separately tested to monitor the degree of extraction of the active ingredients under study.

1.2 Multiple solvent extraction procedure
1.2.1 Separation by Bligh and Dyer lipid extraction procedure
1.2.1.1 Further separation of material obtained in 1.2.1 by thin layer chromatography with different solvent systems
(a) Chloroform-methanol-acetic acid-water.
(b) Chloroform-methanol-concentrated ammonia.
1.2.1.1 Examination of TLC-extracted material as in 1.2.1.1 as to whether it is a single compound or a mixture by
(a) the development of spots in
(i) iodine vapour or
(ii) ninhydrin in water-saturated butanol.
(b) corresponding biological activity of different spot eluates, as obtained from TLC, on isolated guinea-pig ileum.

1.2.2 Separation by gel filtration procedure applied to Folch extraction
1.2.2.1 Attempt at separation of material by gel filtration.
1.2.2.1.1 Sephadex G-10
1.2.2.1.2 Sephadex G-25

Monitoring of gel filtrate samples.

1.2.2.2 Examination of smooth muscle contractile activity of gel filtration aliquots on isolated guinea-pig ileum.

1.2.2.3 Folch procedure extraction of gel-filtrated non-protein active eluates.

1.2.2.3.1 Attempts at further fractionation of Folch-extracted gel filtrate by thin layer chromatography.
(a) development of spot
(b) biological activity of different spot eluates.
2. Attempts at characterization

2.1 Comparison and contrast of smooth muscle contractile material with various autacoids:
   (a) Acetylcholine
   (b) Histamine
   (c) Prostaglandin

Parameters employed:
   (a) Effect of blockers
   (b) Comparison of TLC mobility
   (c) Elution profile with different solvent system in adsorption chromatography.

2.2 Estimation of moieties in purified smooth muscle contractile material as obtained in 1.2.3.3.
   (a) Inorganic phosphorus
   (b) Amino nitrogen
   (c) Amino sugar

2.3 Estimation of contractile activity in terms of phosphatidyl amino nitrogen in isolated guinea-pig ileum.

2.4 Effect of simple physicochemical changes on smooth muscle contractile activity of Gel filtrated solvent extracted material (designated as Substance L).

2.4.1 Heat
2.4.2 Acid-boiling
2.4.3 Alkali-boiling.
2.4.4 Trypsin
2.4.5 Chymotrypsin
2.4.6 Lipase.

3. Pharmacodynamic activity of Substance L (54)

3.1 Experiments concerning the pharmacodynamic study.
3.1.1 Action on cardiovascular system.
3.1.1.1 Experiments concerning blood pressure.
3.1.1.1.1 Effects of intravenous administration of Substance L.
3.1.1.1.2 Influence of Substance L on cat blood pressure alteration by acetylcholine, histamine, 5-hydroxytryptamine and carotid sinus occlusion.

3.1.1.1.3 Influence of various antagonists on SL-induced changes in blood pressure.
   (a) Atropine
   (b) Mepyramine
   (c) Methysergide
   (d) Cyproheptadine
   (e) Propranolol.

3.1.1.1.4 Effect of spinal transection in cat on SL-induced hypotension.
3.1.1.2 Action on heart
3.1.1.2.1 Isolated frog heart
3.1.1.2.2 Isolated guinea-pig heart
3.1.1.2.3 Electrocardiographic changes.
3.1.1.3 Action of substance L on rabbit aortic strip.
3.1.1.4 Action of Substance L on peripheral blood vessels (rat hind quarter perfusion).
3.1.1.5 Effect of substance L on capillary permeability (dye extravasation).
3.1.2 Action of substance L on respiration of cat, guinea-pig & rat.
   (a) On rate
   (b) On amplitude.
3.1.3 Action of substance L on smooth muscle preparations and effect of various antagonists were studied.
3.1.3.1 Action on isolated preparations.
3.1.3.1.1 Guinea-pig ileum
3.1.3.1.2 Guinea-pig colon
3.1.3.1.3 Guinea-pig tracheal chain
3.1.3.1.4 Rat fundal strip
3.1.3.1.5 Rat duodenum
3.1.3.1.6 Rat ileum
3.1.3.1.7 Rat ascending colon
3.1.3.1.8 Rat uterus
3.1.3.1.9 Rabbit jejunum
3.1.3.1.10 Chick - rectum
3.1.3.1.11 Hen rectal caecum
3.1.3.1.12 Hamster stomach.
3.1.3.2 Effect of antagonists on the contractile activity found with 3.1.3.1
   (a) Atropine.
(b) Npyramine
(c) Gyprophedine
(d) Polyphloretin phosphate (PPP).

3.1.4 Search for possible autacoid release and mast cell degranulation
3.1.4.1 Histamine release from guinea-pig chopped lung preparation
3.1.4.2 5-Hydroxytryptamine release from rabbit blood platelet
3.1.4.3 Investigation concerning mast cell degranulation of rat mesentry

3.1.5 Central nervous system and other general changes
3.1.5.1 General behavioural changes
3.1.5.2 Spontaneous motility
3.1.5.3 Body temperature
3.1.5.4 Potentiation of pentobarb sleep

III. Studies concerning enzyme:
1. Phospholipase
   1.1 Crude venom activity
   1.2 Separation of enzyme protein
       1.2.1 Gel filtration (G-100)
       1.2.2 Gel filtration (G-50-150)
       1.2.3 Polyacrylamide gel electrophoresis
   1.3 Measurement of enzyme activity in purified phospholipase fraction in terms of protein content.
   1.4 Elicitation of the extent of purification by
      a) gel filtration
      b) Polyacrylamide gel electrophoresis.
3.5. Location of approximate molecular weight of phospholipase obtained from polyacrylamide gel electrophoresis.