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Research Article

**ISOLATED GOAT ILEUM PREPARATION – AN ALTERNATIVE TO ISOLATED ILEUM PREPARATION FOR THE THREE POINT BIOASSAY OF ACETYLCHOLINE****Madhusudhana Reddy TN<sup>1</sup>, Nagakannan P<sup>1</sup>, Rayappa Hunasagi<sup>1</sup>, Onkaramurthy M<sup>1</sup>, Shivalinge Gowda KP<sup>2\*</sup> and Syed Mansoor Ahmed<sup>1</sup>**<sup>1</sup>Department of Pharmacology, Sree Siddaganga College of Pharmacy, Tumkur, Karnataka, India.<sup>2</sup>Department of Pharmacology, PES College of Pharmacy, Bangalore, Karnataka, India.\*Corresponding Author: [crcpharma@yahoo.com](mailto:crcpharma@yahoo.com)**ABSTRACT**

The objective of the study was to evaluate the use of goat ileum preparation for the estimation of unknown concentration of acetylcholine by three point bioassay. The graded doses of acetylcholine have shown dose dependent increase in contraction of isolated goat ileum. The responses obtained by this tissue preparation were similar to those with guinea pig ileum preparation. The goat ileum can be used successfully in routine pharmacology experiments in place of guinea pig ileum preparation for the three point bioassay of acetylcholine.

**Keywords:** goat ileum, acetylcholine, dose response curve, three point bioassay.

**INTRODUCTION**

Isolated organs and tissue preparations have been extensively used for measuring effects due to receptor – agonist interactions, as well antagonist. The isolated ileum is probably the most widely used model in experimental pharmacology<sup>1</sup>.

Guinea pigs, rabbits and rats are usually the common sources of isolated tissues. Mice are also sometimes used for the purpose, while cats and dogs are too big to be sacrificed just for a piece of tissue<sup>2</sup>. The use of isolated preparations becomes an essential part in experimental pharmacology to understand the effects of a drug, especially in academics.

The Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) is an authority which monitors animal experiments conducted in institutions through ethics committees and is mainly concerned with promoting the humane care of animals used in biomedical and behavioral research<sup>3</sup>. The use of an alternative source definitely serves the purpose of decreasing, if

not completely eliminating, the killing of laboratory animals just for a strip of tissue.

The isolated strips of intestine are the most commonly used smooth muscle preparation, especially the ileum which possesses muscarinic, histaminic, adrenergic, serotonergic and GABAergic receptors. Guinea pig and rabbit intestines are routinely used in preference to other species<sup>[2]</sup>. The presence of muscarinic receptors in goat ileum has been reported. It is both ethical and economical in using goat ileum for this purpose<sup>4</sup>.

The purpose of bioassay is to ascertain the potency of a drug and hence it serves as a quantitative part of any screening procedure. Several methods are used to determine the potency of a drug substance with minimal error. This can be achieved through multiple point bioassay<sup>5</sup>.

The present investigation sought to examine the suitability of the goat ileum for such multiple point bioassay (Three point bioassay).

**MATERIALS AND METHODS**

Fresh ileum of healthy male goat was obtained from a local slaughter house in 250ml warm Tyrode solution and was transported to the laboratory, where it was immediately aerated. The intestinal contents were removed and freed from mesenteric attachments. 2-3 cm portion of ileum was cut and suspended in an organ bath containing Tyrode solution (NaCl<sub>2</sub> – 137, KCl -2.7, CaCl<sub>2</sub> – 1.8, MgCl<sub>2</sub> – 1.0, NaH<sub>2</sub>PO<sub>4</sub> – 0.4, NaHCO<sub>3</sub> – 11.9, Glucose – 5.5) and gassed with 95% O<sub>2</sub> / 5% CO<sub>2</sub>. The tissue was equilibrated for one hour while the temperature was maintained at 35 ± 2°C. 5-7 fold magnification was adjusted with the resting load of 1g. The tissue was washed every 15min<sup>6</sup>.

DRC (Dose Response Curve) was recorded using the standard acetylcholine solution and DRC was also recorded with the test later. In a 'three point' or '2+1' bioassay, two doses of standard and a dose of test which produces intermediate response is selected<sup>7</sup>. Then the three point bioassay was performed by following the Latin square method.

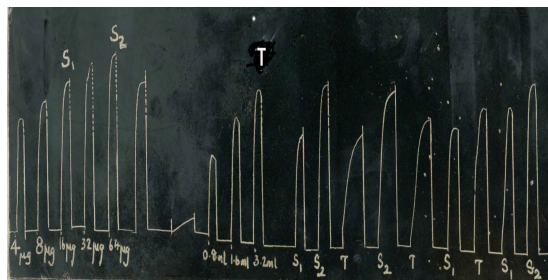
S<sub>1</sub> S<sub>2</sub> T  
 S<sub>2</sub> T S<sub>1</sub>  
 T S<sub>1</sub> S<sub>2</sub>

After recording the response the kymogram was fixed with the fixing solution containing colophony in alcohol. Then the heights of responses were measured and averages were calculated. The averages then substituted in the following formula to get the concentration of the unknown solution.

$$\text{Conc. of Unknown} = n_1 / t \times \text{antilog} \{ (T - S_1) / (S_2 - S_1) \times \log n_2 / n_1 \} C_s$$

Where;

- n<sub>1</sub>= Lower Standard dose
- n<sub>2</sub>= Higher Standard dose
- t = Test dose
- S<sub>1</sub>= Response of n<sub>1</sub>
- S<sub>2</sub> = Response of n<sub>2</sub>
- T = Response of Test (t)
- C<sub>s</sub>= Concentration on Standard



**Fig.1: Three point bioassay of Acetylcholine using goat ileum**

**Table 1: Calculation for three point bioassay of acetylcholine**

Volume (ml)			Response (mm)		
S <sub>1</sub>	S <sub>2</sub>	T	S <sub>1</sub>	S <sub>2</sub>	T
0.8	3.2	3.2	40	54	40
			40	52	42
			47	55	47
<b>Average</b>			42.33	53.66	43.0

**RESULTS**

Acetyl Choline (4µg – 64µg) produced a dose dependent increase in contraction of isolated goat ileum, which was employed as a tool for the three point bioassay (Figure 1 and Table 1). And the results obtained suggest that a preparation such as goat ileum is suitable for the purpose of multipoint bioassay.

**DISCUSSION**

Pharmacology as a subject is particularly hit badly by CPCSEA rules, because the subject is so heavily dependent on animals for research and teaching<sup>8</sup>. And hence an alternative to the commonly used laboratory animals is essential in teaching. Goat ileum preparation has some advantages, such as, easy to suspend in organ bath, has good stability for about 7 – 9 hours, and goat ileum is a tissue that can be easily obtained from a slaughter house and thus avoiding the killing of an animal exclusively for practical purpose. For assay purposes, guinea pig ileum is the choice of tissue, since it gives a steady baseline<sup>7</sup>. This is well obeyed by the goat ileum, hence a suitable tissue for the bioassays, especially the multiple point. However, this preparation cannot be used as a tool to discover new drugs owing to the fact that the receptors present are not fully understood and lack of uniformity of source and maintaining conditions could bring variations in response<sup>4</sup>.

**CONCLUSION**

These results suggests that isolated goat ileum is a suitable preparation for performing routine experiments in pharmacology subject. The preparation also holds some advantage such as easy handling, stability of the preparation is high and economical.

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