- **INTRODUCTION**

BIOASSAY OF SEROTONIN ON RAT STOMACH STRIP BY THREE POINT ASSAY METHOD

OBJECTIVE:-

-To record the dose response curve for serotonin on isolated rat stomach strip preparation

- To plot log vs. response curve for the serotonin standard solution

-To perform bioassay of test sample of serotonin by three-point assay method.

- **EQUIPMENT REQUIRED**

Animal :-	Rat
Drug:-	Serotonin
Instrument:-	Student Organ Bath, kymograph.
Physiological salt solution:-	Krebs-Henseleit solution

- **PRINCIPLE**

The bioassay of serotonin using the rat stomach strip is based on the principle that serotonin (5-hydroxytryptamine, 5-HT) induces a contractile response in smooth muscle, which can be measured to determine its potency and concentration. The three-point assay method is a comparative bioassay technique that quantifies the unknown concentration of serotonin by comparing its effect to that of a known standard.

In this method, three different doses of the test sample and standard serotonin are selected, ensuring they produce responses within the linear portion of the dose-response curve. The contractile responses are recorded, and the unknown concentration is determined by interpolation using the standard response values. This method assumes a direct proportionality between concentration and response within the selected dose range.

The rat stomach strip is chosen as the biological model because it contains serotonin-sensitive receptors (5-HT receptors) that mediate contraction. The accuracy of this assay depends on maintaining controlled experimental conditions, including temperature, pH, and proper tissue handling.

PROCEDURE:

1) Sacrifice rat and isolate stomach ad prepare tissue by taking stomach strips and place it in krebs-henseleit solution

2) Setup organ bath and tie ends of stomach strip to the aeration tube and isotonic lever.
3) Stabilize the tissue and allow it to withstand in the physiological salt solution for 30minutes. And change the solution at intervals of 10minutes.
4) Introduce serotonin and take baseline at 30sec, record concentration using isotonic writing lever.
5) The contact time of tissue maintained should be of 30seconds.
6) Wash the tissue 3times after administering dose in graded response with washing time of 60sec.
7) Total time cycle should be maintained for 240sec.
8) Repeat the process of introducing serotonin three times.

CALCULATION & INTERPRETATION:

Potency of test solution = (S1/T1) x antilog [(T1 – S1) / (S2-S1) x log (S2/S1) x Conc. Of standard

Where:

S_1 = Height of contraction produced by standard dose S_1
T = Height of contraction produced by standard dose T
S_2 = Height of contraction produced by standard dose S_2

CONCLUSION

The bioassay of serotonin on the rat stomach strip using the three-point assay method successfully determined the potency and concentration of serotonin based on its contractile response. The method provided a reliable comparison between the test and standard serotonin samples, confirming the responsiveness of the rat stomach strip to serotonin stimulation. The results demonstrated a dose-dependent contraction, validating the sensitivity of the assay. This technique is useful for quantifying serotonin activity in pharmacological studies and assessing the effects of serotonin receptor modulators.

IDEAL OBSERVATION

Sr. No	Conc. Of Serotonin (µg/mL)	Amount Added in Organ Bath		Conc. of Ach in µg/mL (in organ bath contains 20ml solution)	Response (in m m)	%Response
		In mL	In µg			
1.	10	0.1	1	0.05	4	19.04
2.	10	0.1	1	0.05	4	19.04

3.	10	0.2	2	0.1	7	33.33
4.	10	0.4	4	0.2	12	57.14
5.	10	0.8	8	0.4	15	71.42
6.	10	1.6	16	0.8	21	100.00
7.	10	3.2	32	1.6	21	100.00

Test solution of Serotonin		
Sr. No.	Amount Added to organ bath (ml)	Response (mm)
1	0.2	6
2	0.4	9
3	0.8	14
4	1.6	18

$S_1 = 0.2$
$t = 0.4$
$S_2 = 0.4$

Conc. of standard is 10 µg/ml

Latin sequence design

[s_1 t s_2]

[t s_2 s_1]

[s_2 s_1 t]

Sample	Dose(mL)	Conc.(µg)	Height of conc. (mm)	Mean Height
S_1	0.2	2	7	$S_1 = 7$
S_1			6	
S_1			8	
t	0.4	?	9	T = 9
t			8	
t			10	
S_2	0.4	4	12	$S_2 = 13$
S_2			13	
S_2			14	

Potency of test solution = $(s_1 / t_1) \times \text{antilog} [(T_1 - S_1) / (S_2 - S_1) \times \log (s_2 / s_1)]$ X conc. of standard

Where:

- S_1 = Height of contraction produced by standard dose s_1
- S_2 = Height of contraction produced by standard dose s_2
- T_1 = Height of contraction produced by test dose t_1

0.628 x 10

6.28 µg/ml

RESULT:
The bioassay of serotonin on the rat stomach strip using the three-point assay method demonstrated a dose-dependent contractile response. The unknown concentration of serotonin was determined by comparing the responses of the test sample to the standard. The calculated potency ratio indicated that the test sample exhibited a contractile effect similar to that of the standard serotonin, confirming the accuracy of the assay. The results validate the effectiveness of the three-point assay method in determining serotonin activity in smooth muscle tissues.

DISCUSSION:
The bioassay of serotonin on the rat stomach strip using the three-point assay method effectively quantified the contractile response of smooth muscle to serotonin stimulation. Serotonin (5-HT) plays a crucial role in gastrointestinal motility by acting on 5-HT receptors, leading to muscle contraction. The rat stomach strip was chosen for this assay due to its high sensitivity to serotonin, making it a reliable biological model for assessing its pharmacological activity.

The three-point assay method is based on the principle that responses to an unknown concentration of serotonin can be interpolated by comparing them with known standard doses. This method ensures accuracy and minimizes errors caused by variations in tissue sensitivity. The observed dose-dependent contractions confirmed the presence of serotonin-sensitive receptors and validated the effectiveness of the assay.

The results demonstrated that serotonin produced a significant contractile effect, and the calculated potency ratio provided an estimate of the unknown concentration. The linear relationship between dose and response within the selected range confirmed the validity of the bioassay. Factors such as tissue viability, experimental conditions (temperature, pH, oxygenation), and proper calibration of doses were crucial in ensuring accurate results.
This bioassay is essential for pharmacological research, particularly in studying serotonin's effects on smooth muscle and evaluating drugs that modulate serotonin receptors. Future studies can explore the use of receptor antagonists to further characterize the receptor subtype involved in serotonin-induced contractions.