

Bioassay of D-tubocurarine

Dr. Rajesh Choudhary
M. Pharm. (Pharmacology), Ph. D.

 www.youtube.com/pharmacologyconceptsbyrajeshchoudhary

 www.pharmacyconcepts.com

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D-tubocurarine



Fig. *Chondrodendron tomentosum*. main source plant of 'Tube Curare' and principal source of **D-tubocurarine (DTC)**.

✓ **Curare** is a common name for various plant extract alkaloid **arrow poisons**



Source: <https://metode.org/issues/article-revistes/from-the-jungle-to-the-operating-theatre.html>

D-tubocurarine



- ✓ **D-tubocurarine** is a non depolarizing potent skeletal muscle relaxant
- ✓ It is competitive and reversible antagonist of Nicotinic Ach Receptor especially N_M receptor
- ✓ It blocks the N_M receptor at neuromuscular junction and cause the relaxation of skeletal muscle
- ✓ In the mid-1900s, it was used in conjunction with an anaesthetic to provide skeletal muscle relaxation during surgery
- ✓ Overdose may leads to muscular paralysis that can be observed during Bioassay of **D-tubocurarine**

Bioassay D-tubocurarine



Bioassay: Biological assays are a set of techniques for estimating the **Potency** or **Strength** of an “agent” by Using the “response” or “effect” on biological system or experimental living subjects.

Basic Principle: Potency of test D-tubocurarine is determined by comparing its specific activity with standard preparation of D-tubocurarine by using specific biological assay method.

Activity: Skeletal muscle relaxant

Bioassay D-tubocurarine

Bioassay Methods:

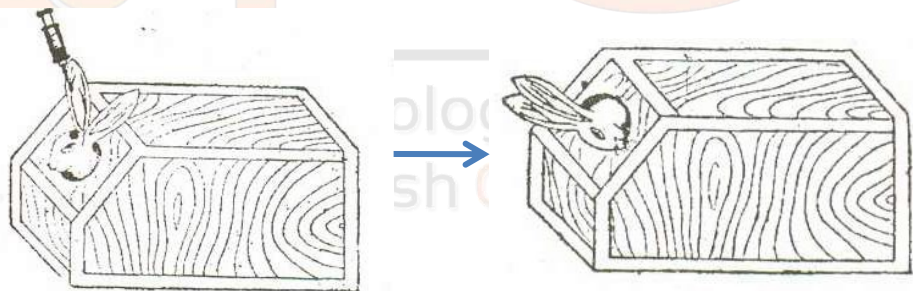
1. Rabbit head drop model
2. Frog's Rectus Abdominis muscle Preparation

Pharmacology Concepts
By Rajesh Choudhary

Bioassay D-tubocurarine

1. Rabbit head drop model

Principle: Standard/test sample of d-Tubocurarine hydrochloride is injected into the marginal ear vein of a rabbit till neck muscles are completely relaxed and animal cannot hold its head up. The total amount of test sample required to produce the endpoint is compared with the total amount of the standard sample required to produce similar endpoint





Bioassay D-tubocurarine

1. Rabbit head drop model

Animal: Healthy rabbits (2-2.5 kg) are used in this test. Rabbit should be habituated in experimental environment before the experiments.

Procedure:

Step 1. Rabbits are divided into 2 group each group contains 4-5 animal.

One group is treated (injected) with standard D-tubocurarine solution (0.012% w/v in saline) and another is treated with test solution.

Step 2. Each rabbit is placed in a holder with its head protruding outside. The head should be freely movable



Bioassay D-tubocurarine

1. Rabbit head drop model

Step 3. d-Tubocurarine solution (Standard/Test) is infused/injected at a constant speed (0.4 ml/min) by infusion apparatus through the marginal vein

Step 4. Infusion is continued till the rabbit will not be in a position to hold its head erect or there will be no response by focusing light on the eyes.



Bioassay D-tubocurarine

1. Rabbit head drop model

Step 5. Rabbits recover immediately from the effect of curare. If respiratory embarrassment is observed during the experiment animal should be treated with neostigmine methyl sulphate (0.05 mg.) and atropine sulphate immediately through the marginal ear vein.

Step 6. To minimize the biological error Cross-over test is carried out. Those rabbits which received the standard will be given test sample and vice versa.

Step 7. Mean dose of the test sample that required to head drop is compared with the mean dose of standard preparation.

Bioassay D-tubocurarine



1. Rabbit head drop model

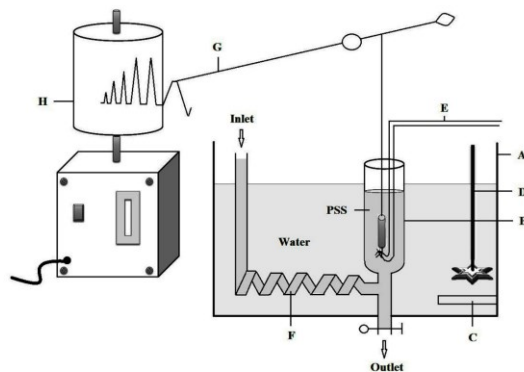
Calculation:

Conc of Unknown =

$$\frac{\text{Threshold dose of std}}{\text{Threshold dose of test}} \times \text{Conc. of Std}$$

Bioassay D-tubocurarine

2. Frog's Rectus Abdominis muscle Preparation



Bioassay D-tubocurarine

2. Frog's Rectus Abdominis muscle Preparation

Principle: The potent skeletal muscle relaxant inhibits the contractile response of Acetylcholine. The inhibitory effect of test D-tubocurarine against Ach is compared with the standard D-tubocurarine.

Step 1. Rectus abdominis muscle is isolated from frog and placed in frog's ringer solution (PSS).

Step 2. Tissue is mounted on organ bath contain PSS and oxygenated with 95% O₂ and 5% CO₂

Step 3. The muscle is stabilized for 30 to 40 min to get proper response and the response are recorded by using isotonic frontal writing lever with 1 g of tension.

Bioassay D-tubocurarine

2. Frog's Rectus Abdominis muscle Preparation

Step 4. After stabilization of tissue preparation, cumulative dose response curve of acetylcholine is recorded without D-tubocurarine.



Normal dose response



Cumulative dose response

Step 5. After getting the cumulative dose response curve of acetylcholine, the tissue is washed out (2-3 times) and then Standard drug is added in organ bath solution and again record the cumulative dose response curve of acetylcholine. And calculate the % inhibitory response of Std solution

Bioassay D-tubocurarine

2. Frog's Rectus Abdominis muscle Preparation

Step 6. After getting the cumulative dose response curve of acetylcholine with Std, the tissue is washed out (2-3 times) and then test drug is added in organ bath solution and again record the cumulative dose response curve of acetylcholine. And calculate the % inhibitory response of test solution

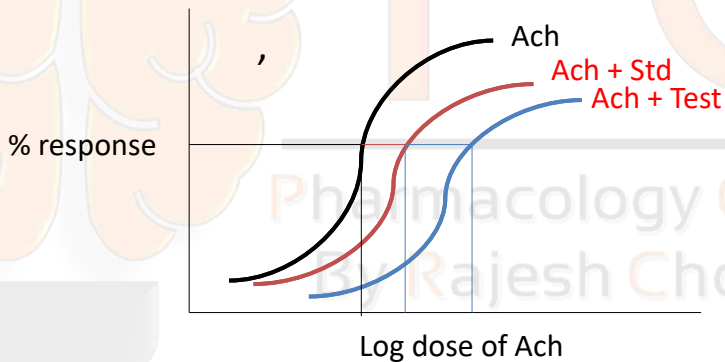
Step 7. Plot the cumulative response curve and calculate potency of test drug by compared with standard



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2. Frog's Rectus Abdominis muscle Preparation

Graph:



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