Bioassay of Digitalis

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Digitalis purpurea (Foxglove)

DIGITLIS:

- ✓ It is a Cardiac Glycoside
- Digitoxin & Digoxin- Active Chemical constituents
- Digitalis (Digitoxin & Digoxin)- are the cardiotonic drug, which are used in the treatment of CHF.
- Digitalis mainly affects the ion transport system
 (Na+K+ATPase pump) and Electrophysiology on
 myocardial tissues and improve the cardiac
 performance
 - At high dose it causes cardiac arrest



Bio: Living system

Assay: Test

Bioassays: Test of a substance on living system.

Principle: Potency of the test sample of Digitalis is compared with that of the standard preparation of Digitalis by determining the action on the cardiac muscle like cardiac arrest.

Standard Preparation and Unit: The standard preparation is a mixture of dried and powdered digitalis leaves (1 U = 76 mg).



Extract Preparation of Standard and Test :

- Exact amount of the powder is extracted with dehydrated alcohol in a continuous extraction apparatus for six hours.
- The final extract should contain 10 ml (5 ml Alcohol + 5 ml water) per 10g of digitalis powder.
- ✓ And it should be stored in between 5 to -5 °C.

Preparation of Standard and Test solution

Standard and test sample extracts are diluted with normal saline (1g of digitalis powder is diluted to 80 ml)



Animal Models:

- 1. Guinea Pig Model (End point method)
- 2. Cat Model (End point method)
- 3. Pigeon Model.

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1. Guinea Pig (End Point Method):

- Adult Guinea pigs are used in this method.
- Experimental procedure should be approved from IAEC (Institutional Ethics committee) of the concern department prior to the experiments on animal

Experimental procedure

- 1. A guinea pig is anaesthetized with suitable agent and dissected on the operation table
- 2. The jugular vein is cannulated by venous cannula for the infusion of test/standard substance.
- 3. A pin is inserted in the apex of the Heart to observe the heart beats by up and down movements of the pin.
- 4. Thereafter standard Digitalis is infused through venous cannula until the heart is arrested in systole.



Experimental procedure

- 5. The amount/Voulme of extract required to produce lethal cardiac arrest is noted as the lethal dose of the extract.
- 6. Another sets (5-6) of experiments are performed to determine the lethal dose of standard.
- 7. Similar procedure is applied for the test sample of digitalis and determine the lethal dose of test.
- 8. And finally, the potency of test is calculated against standard and expressed as U/g of guinea pig

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2. Cat model (End Point Method):

- ✓ Adult cats are used in this method.
- Experimental procedure is almost similar as guinea pig model.
 End point:
- The moment the heart stops beating and blood pressure falls to zero, the volume of fluid (Std/test) infused is noted down
- ✓ And calculate the potency

Conc. of Test = (Threshold dose of Std/ Threshold dose of test) x Conc. of Std.



3. Pigeon method

- 1. Minimum 5-6 Pigeon are used for testing each sample.
- The weight of the heaviest pigeon should not exceed twice the weight of the lightest pigeon.
- 3. Food is withheld 16-28 hours before the experiment.
- Pigeons are divided on the basis of their sex, weight and breed, into two groups.
- 5. They are anaesthetized with anesthetic ether or any suitable agent
- 6. One side of the wing is dissected and the alar vein is cannulated by means of a venous cannula.



3. Pigeon method

- 7. The test sample and standard sample is infused through cannula.
- 8. In pigeons, stoppage of heart is associated with a characteristic vomiting response called 'emesis'.
- 9. The milk from the crop sac of pigeons is being ejected out. This may be taken as the end point response of digitalis.
- 10. The lethal dose per kg. of body weight is determined for each pigeon.
- 11. The potency of the test sample is determined by dividing the mean lethal dose of standard by the mean lethal dose of the test sample.



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