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PHARMACOLOGICAL ASPECTS IN DRUG STANDARDISATION

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In medical practice, it is very much essential to standardise the dose of a drug which when administered clinically to a patient should have produced a desirable degree of therapeutic effect without having any untoward or side effects. i.e. the amount of a drug administered either orally or injected parentally should be adjusted as such that it should neither be too low which fails to produce any therapeutic effect or too high to produce its over effects which are undesirable. Therefore, standardisation of a dose of a drug is very much essential.

Standardisation of a drug can be done by

Chemical assay, Microbiological assay and Bioassay

Chemical assay

It is done mainly for pure chemical substances where it is possible to measure the actual quantity of a substance in a drug; Various tests employed are: T.L.C., H.P.L.C., U.V., I.R., Atomic Absorption Spectrophotometery, Mass Spectroscopy, NMR etc.

Microbiological assay

It is done for determining the concentration of an antimicrobial agent necessary to kill the desired microbes. The tests employed are: Turbidimetric or tube assay method, Cylinder - Plate or cup-plate method

Bioassay

Standardisation of a drug by this method is done only when:

- There is no suitable chemical method for standardising the dose of a drug. e.g. Digitalis leaves, Insulin. Corticotrophin, Oxytocin, Vasopressin, Antitoxins etc.
- When a particular racaemic form of a chemical drug is more biologically active than the other.
- When it is possible to observe the characteristic reproducible response of a drug on a living tissue.

The principle of bioassay is to compare how much of a sample of a drug being tested produces the same biological response as a given quantity of a standard preparation, provided that both sample and standard are tested in identical conditions in all respect of time, environmental factors and biological media used. For a typical pharmacological test, following criteria is needed :

A. A stimulus (drug)
B. A subject (animal, animal tissue, bacterial culture)
C. Intensity of stimulus applied to the subject (i.e. dose-weight. concentration, or volume)
D. Observed response (increase or decrease in heart rate, B.P., respiration, blood sugar etc)

Bioassay has some limitations too. Actual potency of a sample cannot be judged; only approximate potency with a confidence limit (95% or 99%) can be calculated by using statistical analysis. (SDM, SEM, Students 't' test, X^2 test, analysis of variance)

Bioassay of Insulin

Potency of an unknown sample of the Insulin is determined by comparing its potency with a standard preparation of the insulin.

Testing Methods

Method-1

Animal used - Mice

Preparation of a standard solution of Insulin:

- Prepare a standard solution containing 40 U/ml of insulin (crystalline insulin containing 24 U/mg) in acidified normal saline solution (pH 2.5).

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Experimental

- Take 96 mice, deprive them for food for 12 hrs and divide them into 4 groups.
- Make 2 dilutions of standard preparation (60 and 30 mU/ml) and of test sample.
- Inject insulin solution in same volume (> 0.5ml) s.c.
- Keep the mice at room temperature 29-35°
- Record the observations (mice-dead, convulsed or lie still for < 2-3 sec).
- Compare the results

Method-2

Animal used - Rabbits

Preparation of a Standard solution of Insulin

- Prepare standard solution of insulin containing 1 and 2 U/ml. in acidified normal saline solution (pH 2.5).

Experimental

- Take 24 rabbits weighing > 1.8 Kg.
- Keep them for a week.
- Deprive them for food for 12-18 hrs
- Divide them into 4 groups.
- Inject 0.5 ml in each rabbit.
- Collect 5 ml blood samples after one and two and half an hour after injection.
- Determine blood glucose level.
- Compare the result.

Bioassay of Corticotrophin

Principle

Potency of Corticotrophin in an unknown sample is determined by comparing its ascorbic acid depletion property for adrenal cortex with that of a standard preparation of corticotrophin.

Standard preparation and its unit

It is the quantity of dry corticotrophin approved by Government and preserved for record. One unit of the standard is equivalent to 0.88 mg of the drug.

Testing Methods

Method - 1

Animal used - Rats (100-200 g, body weight of individual animal in the test group should not vary > 15g).

Experimental

- Hypophysectomise the rat under anaesthesia.
- Give 5% dextrose in saline solution in addition to food and water.
- Keep the animals in a quiet room at a constant temperature of 24-27°.
- Carry out the test procedure between 18-36 hrs after operation.
- On the day of test, weigh the animal and divide them into 6 groups of 8-10 each.
- Choose 3 doses (1.0, 0.5 and 0.25 U/100g body wt.) of standard preparation and 3 doses of unknown sample and inject s.c. or i.v.
- Remove both adrenals from anaesthetised animal after 3 hr. of s.c. injection (or 1 hr. in i.v.).
- Measure the ascorbic acid content and calculate its depletion.

Standardisation of Antibacterial Activity of Homoeopathic Drugs

Minimal bactericidal concentrations (MBC) of mother tincture for three different samples against Staph. aureus has been determined by Tube dilution method. Dilutions (1:20 to 1:640) were prepared in 1 ml nutrient broth which were then inoculated with suspensions of Staph. aureus. The MBC was recorded as the highest dilution to show no bacterial growth. This has been taken from British Homoeopathic Journal Vol. 81 : 78-81, 1992.

Name of Drug	Manufacturer A	B	C
Anacardium orientale	160	40	64
Aralia racemosa	-	320	40
Benzole		20	20
Berberis vulgaris	-	-	40
Camphor	160	20	-
Chimaphila umbellata	40	40	40
Chionanthus virginaca	40	-	20
Cupressus sempervirens		20	40
Drosera rotundifolia	-	20	20
Eucalyptus globulus	160	80	160
Glycyrrhiza glabra	-	80	40
Guaiacum	40	80	40
Hydrastis canadensis	40	160	160
Inula Helenium	-	80	40
Propolis	-	80	160
Sequoia	-	20	80
Spirea ulmaria	40	-	40
Thuja	20	-	20

Quality Control of Homoeopathic Medicines

Pharmacological screening of homoeopathic drugs

Pharmacological screening is to be carried out

- For determining the acute, subacute and chronic toxicity of a new drug or a new preparation
- For carcinogenic effect
- For teratogenic effect
- For determining the biological activity (CNS, CVS, antimicrobial, antiparasitic, antiviral, anticancer, immunosuppressants etc) of a new drug
- For determining maximum therapeutic dose and the margin of safety of a drug
- For determining the onset and duration of action of a drug.

Toxicity Testing

Toxicity testing of finished product mainly of mother tincture or upto 6X triturations should essentially be carried out as these are the preparations which contain sufficient quantity of active chemical constituents which may produce toxic effect if administered in material doses.

General Toxicity test

- Take 5 healthy mice (17-22g)
- Administer (or inject) the drug to each animal and observe the effect for 48 hrs.

Interpretation

The drug passes the test if none of the mice dies within the time interval specified. If one of the animal dies, repeat the test. The drug passes the test if none of the animal in second group dies within the time interval specified.

Test for Pyrogens

Principle

It involves measuring of rise in body temperature of rabbits after intravenous injection of a test drug.

Caution

- Use healthy rabbits (body wt. > 1.5 Kg)
- Do not repeat test on the same animals before 48 hrs
- Give a rest period of at least 2 weeks if body temperature rises < 0.6°C in previous test.

Preliminary (sham) test

- Condition the animals for 3 days
- Withhold food overnight and, food and water during test.
- Inject 10 ml/kg pyrogen free saline solution
- Record body temperature at every half an hour interval (90 min before and upto 3 hrs after injection).

Note: - Animals showing rise in body temperature 0.6°C or more should not be used for main test.

Main test

- Use 3 rabbits for each group
- Inject each drug sample i.v. in doses (> 0.5 ml/kg and < 10 ml/kg)
- Record the rise in body temperature as earlier

Interpretation

- If sum of the rise in body temperature of 3 rabbits does not exceed 1.4°C and if the rise in body temperature of an individual rabbit is less than 0.6°C then the sample passes the test.
- If any of the above value is higher then repeat the test with 5 more rabbits
- If sum of the rise in body temperature of 8 rabbits is <3.7°C and if out of 8 rabbits not more than 3 rabbits show individual response of 0.6°C then the sample being examined passes the test.

Sterility Test

In Homoeopathy sterility test is done for those preparations which are prepared in aqueous solution and are used topically mainly for eyes.

- The test for sterility are designed to reveal the presence of micro-organisms (bacteria, fungi, yeasts) in the samples used in the test.

Principle

The tests are based on the principle that if bacteria or fungi are placed in a medium which provides nutritive materials and water, and kept at a favourable temperature, the organism will grow and their presence can be indicated by a turbidity in the originally clear medium.