EVALUATION OF CRUDE DRUGS

Disclaimer

All the content material provided here is only for teaching purpose.

6.Biological Evaluation

- It is employed when the drug cannot be evaluated satisfactorily by chemical and physical methods.
- In this method, the response produced by the test drug on a living system is compared with that of the stranded preparation.
- Such an activity is represented in units as International Units (I.U).Dose is termed as International units IU

- Digitalis 1IU=76mg of standard
- Vit-A 1IU=0.344 of standard
- Vit-D 1IU=0.025of standard

Indication of Biological Evaluation

- When the chemical nature of the drug is not known but is has an biological action.
- When chemical methods are not available.
- When the quantity of the drug is small and so it cannot be evaluated chemically.
- Drugs which have different chemical composition but same biological activity.
- Example: Cardiac glycosides arte evaluated by this method on cats, frogs or pigeons.

SIGNIFICANCE

1. The method is generally used when standardization is not done satisfactory by chemical or physical methods
2. When the quantity of the drug /sample are very less then the drugs are evaluated by biological methods.
3. These methods are performed on living animals, isolating living organ and tissue, animal preparation, and micro-organism (Bioassay)

METHODS OF STUDIES

1)Toxic----animals are used
2)Symptomatic-----animals are used
3)Tissue-------isolated tissue is used

- To estimate potency of drug
- With entire animal or with tissue
- To conform therapeutic activity

EVALUATION OF HEAPATOPROTECTIVITY

ANIMALS USED: Male and Female Albino rats

Heapatotoxicity indused by: Chemicals

Industrial pollutants ccl4

Drugs (paracetamol,rifampicin)

PARAMETERS FOR ESTIMATION

1.PHYSIOLOGICAL—HEXOBARBITAL HYPNOSIS

2.BIO-CHEMICAL—SERUM ESTIMATION ENZYMES LIKE

SGOT(serum glutamic oxaloacetic transaminase)

SGPT (serum glutamic pyruvic oxaloacetic transaminase)

3.BLOOD CHELESTEROL,TRIGLYCERIDES LEVELS

4.HISTOPATHOLOGICAL METHODS (liver tissue necrosis)

for testing cultured heaptocytes are used for In-vitro studies

Evaluation of Hypoglycemic activity

deficiency of glucose in the bloodstream.

- Traditional Diabetic drugs -Momordica charantaka,Fenu greek,Gudmar.
- Diabetis is induced in animals by Alloxan & Streptazocin
- Alloxan cause necrosis of pancreatic islet-B cells which shows 180-250mg/ml fasting blood glucose levels
- Streptazocin cause formation of streptomycin they produce cytotoxic nitrourcido glucopyranose which cause diabetes

ANIMALS USED: Rabbits,Rats,Mice—(4 to 7 days)

Dose :rats--80mg/kg ,mice—150mg/kg of streptazocin

single oral injection 140-180 mg/kg of alloxan for rabbits at marginal ear vein for 7 days

for rats and mice intraperitoneally 2 days

Insulin levels are noted by tests like RIA.ELISA.

Evaluation of Anti-inflammatory activity

Inflammation is caused by mechanical,infections,auto-immune

Types of inflammations:Rhemated arthritis,gout,dysmenorrhoea

PRINCIPLE: Anti-inflammatory activity is reduction of local edema induced in rat paw by injecting irritant or inflammatory substance

Inflammation is induced by carrageenan and croton oil

Methods 1: Carrageenan is a muco-polysaccharide isolated from sea moss which induces inflammation by giving through intraperitoneally saline in a dose of 0.1 ml.

animal is treated with herbal extract given orally (antagonist)

Volume of paw is measured five times with plethysmo meter

Methods 2: Here albino rats or mice are used, edema is produced pinna of ear with croton oil(1 ml/ear)

After induce herbal extract is added to the same area

Edema is measured by using verniercallipers and record the changes

0---no effect

+ve---slight

++ve---pronounced

Evaluation of Anti fertility activity

Abortificient activity, contraceptives

Traditional drugs like embelin from embilica and gossypol from gossypium produce abortificient activity

Types of Anti fertility evaluations

In-females : Destruction of zygotes &prevention of ovulation

In-males : estimation of Spermicidal activity & Anti-androgenic activity

Protocols for anti- spermatogenic activity

After acclimatization male rats are feed by herbal extract for 60 days

↓

Between 12 day and 15 day & 60 th day male rats are mated with female (morphological weight of rat noted)

↓

Histopathlogical study of sperm

↓

RESULT If No-fertilization b/w 12-15 days means functional sterility, after 56 days means anti-spermatogenic

Spermicidal activity (in-vitro)

Take human semen on a slide and add herbal extract and sorensens phosphate buffer

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Microscopic examination

↓

Identification of Immobility means spermicidal

Protocols for anti-fertility in female rats

Acclimatized 8-10 days (I-group)

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Vaginal smears are taken to measure cycle

↓

Animals are feed by herbal extract

↓

(oestrogenisity leads increase weight of uterus / cornifiction of vagina)

↓

Further the rats are mate with males

↓

(mating detected by smears)

↓

Ovulation is absent due to contraceptive activity)

↓

In case of fertilization ova are examined to see the transportation and implantation

↓

Acclimatized 19 days (II-group)

↓

- If weight gain contraception
- If bleeding abortion

Testing of anti-ulcer activity

Causes of ulcer -- improper diet, alcohol consumption,stress,drugs(NSAIDS)

Traditional drugs like liquorice,atropine,hyoscine and in less extent Gafaranate extracted from cabbage juice shows anti ulcer effect

Chemical used to induce ulcer: Alcohol 1ml/kg orally

Aspirin -200mg/kg orally

Stress induced: animals are immobilized in cylindrical cage

Animals are devided in to 3 groups

1.Those treated with normal saline

2.Those treated with ulcerogenic solution

3.Strss produced

Animals are sacrificed after inducing

0-no damage	0-absence
1.redness of mucosa	1.slight
2.Erosin of mucosa	2.One ulcer 5mm length
3.ulceration	3.More than one
	4.One above 5 mm
	10.Total ulcerations

All the groups are treated with herbal extracts and standard is treated with ranitidine

Stomach or duodenum is isolated

Organs are opened to know ulcer effect and gastric juice is measured and ulcer effect express in table

Evaluation of Neuropharmacologocal activity

Testing of herbal drugs on CNS&ANS

Drugs and actions actions on

CNS:cocaine,cannabis,morphine(stimulants,tranquilisers)

METHODS FOR TESTING:

1.LOCOMOTAR ACTIVITY-----ACTIVITY CAGE(locomotion count is noted)

2.LOCOMOTAR CO-ORDINATION-----ROTATING ROD

3.PENTETRAZOLE CONVULSION IN MICE 80-120 mg/kg- intraperitonelly

a)time of onset of convulsions

b)number of convulsions

c)mortality rate

4.BARBITURATE SLEEPING TIME stimulant or depressant effects on CNS

Phenobarbitone ----55mg/kg

Hexobarbitone -----33mg/kg

➢Due to drug treatment if ACH type contractions are produced, persist even after giving a dose of histamine H1-receptor antagonist, then it may be indicated that it is a cholinergic drug. it can be verified by blocking effect with atropine.(it does not distinguish between muscarinic and nicotinic receptor activity.

➢If contractions caused by herbal drug are blocked by ganglion blocking agent then it may be indicated that herbal drug is nicotinic receptor mechanism .

➢Besides cholinergic activity the contractions occurred due to drug may be because of its activity at other receptors like histamine.

Microbiological Assays

Drug substances suppress or influence the growth of micro organism are generally analyzed by microbiological method.(Anti- biotics, Vitamins)

1.Cylindrical plate method

Assay of antibiotics is based on measurement of the diameter of microbial growth inhibition surrounding the cylinders containing various dilutions of test compound which are placed on surface of solid nutrient medium .

2.Turbidimetric method

Based on inhibition of microbial growth as indicated by measurement of turbidity(transmittance) of suspension of a suitable micro-organism in a fluid medium, to which have test compound. changes in transmittance is compared wit standard known compound.

QUALITY CONTROL OF HERBAL DRUGS

WHO guide lines to ensure quality of herbal drugs with modern techniques

Monograph guide lines

1.Monograph title

A) Botanical

a)sensory evaluation
b)foreign matter----(should free from it if possible)
c)microscopy

B)Physico-chemical

a)TLC
b)Ash----total, acid insoluble, water soluble
c)Extractive matter---in hot water, cold water, ethanol
d)water content and volatile matter----LOD
e)volatile oil-------steam distillation

C)Pharmacological

a)Bitterness value—units equal to bitterness of std.solu of quinine hydrochloride
b)Hemolytic activity----on Ox blood by comparison with std.ref.saponin
c)Astringency---Fraction(tannin)that binds to std.hide powder
d)swelling index---in water
e)foaming index---foam height produced by 14 gm material under specified conditions

D.Toxicological:

a)Pesticide residue---chlorides, phosphorus estimation

b)Arsenic—strain produced on HgBr2 paper in comparison to std.stain

c)Heavy metals---cadmium, lead

$$\text{Maximum residue limit} = \frac{\text{BW X ADI X Extraction factor}}{\text{safety factor 100 X MDI}}$$

ADI = avg.daily intake,

BW = body weight

MDI = mean daily intake

E.Microbial contamination:

a)total viable aerobic count

pathogen:*E.coli,*

salmonella,P.aerogenosa,S.aures

Aflatoxins:by TLC using std.Aflatoxins (B1,B2,G1,G2) mixtures----totally free

F.Radioactive contamination:

As per recommendations of international atomic energy agency IAEA

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