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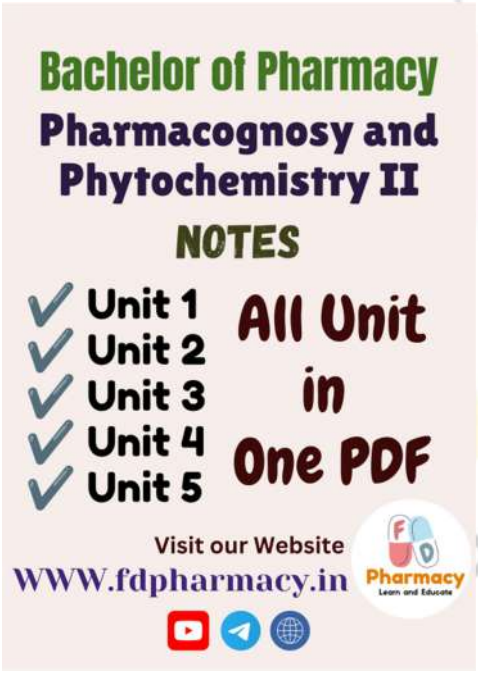
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PHARMACOLOGY - II

UNIT 5

TOPIC :

- **Bioassay**

  a. Principles and applications of bioassay.

  b. Types of bioassay

  c. Bioassay of insulin, oxytocin, vasopressin, ACTH, d-tubocurarine, digitalis, histamine and 5-HT

Bioassay

- Bioassay is the quantitative or qualitative measurement of the biological activity of a substance (drug, hormone, or toxin) by observing its effect on living cells, tissues, or organisms. It is used when the chemical assay is insufficient to measure the pharmacological activity.

Principles of Bioassay

1. Biological Response Correlation:
   - The effect of a drug on a living system is proportional to its concentration or dose.
2. Specificity:
   - The test system must be specific for the drug under study to avoid interference by other substances.
3. Quantitative Measurement:
   - Effects are measured using endpoints (e.g., contraction, secretion, BP change, enzyme activity) which are dose-dependent.
4. Standardization:
   - Comparison is made with a standard drug of known activity to estimate the potency of the test drug.

Applications of Bioassay

1. Potency Determination:
   - Measuring strength of a drug preparation (e.g., insulin, digitalis).
2. Standardization of Hormones:
   - Ensures consistent biological activity in hormonal preparations.
3. Drug Development:
   - Screening new compounds for biological activity.
4. Quality Control:
   - Detect contaminants or degraded products affecting activity.
5. Research:
   - Study receptor mechanisms and pharmacodynamics.

Types of Bioassay

1. Qualitative Bioassay

- Determines the presence or absence of a substance.
- Example: Testing oxytocin in uterine tissue.

2. Quantitative Bioassay

- Measures exact biological activity or potency.
- Methods:
    1. Direct or Simple Bioassay: Single preparation; compare dose–response with standard.
    2. Indirect or Parallel Line Bioassay: Uses multiple doses; compares slope of test vs. standard dose–response curves.

3. In Vivo Bioassay

- Performed in living animals.
- Examples:
    - Insulin → blood glucose lowering in rats
    - Oxytocin → uterine contraction in rats/rabbits

4. In Vitro Bioassay

- Performed on isolated tissues/organs or cells.
- Advantages: Less animal use, more controlled environment.
- Examples:
    - Isolated ileum for histamine
    - Frog heart for digitalis

BIOASSAY OF DRUGS

Insulin

Principle:

Insulin lowers blood glucose. Its activity can be measured by the hypoglycemic effect in animals or glucose uptake in tissues.

Common Bioassays:

1. In vivo: Albino rats or rabbits
    - Procedure: Inject insulin → measure blood glucose drop at intervals (30, 60, 120 min).
    - Remarks: Blood glucose measured by glucose oxidase or colorimetric methods.
2. In vitro: Rat diaphragm or fat pad
    - Measures glucose uptake in isolated tissue.

Notes:

- Standard insulin is used as reference.
- Care must be taken to maintain animal fasting state for accuracy.

Oxytocin

Principle:

Oxytocin stimulates uterine contractions in gravid animals or cervical tissue.

Common Bioassays:

1. In vitro: Isolated uterine strip of rat or guinea pig (pregnant)
    - Procedure: Place uterine strip in organ bath → add oxytocin → record contractions using isotonic or kymograph method.
    - Standard: International Unit (IU) of oxytocin.
2. In vivo: Pregnant guinea pig
    - Observe labor-inducing effect or number/force of uterine contractions.

Remarks:

- Sensitive to temperature, pH, Ca^{2+} concentration of the organ bath.

Vasopressin (ADH)

Principle:

Vasopressin produces pressor effect (↑ BP) and antidiuretic effect.

Common Bioassays:

1. In vivo: Rat tail blood pressure method
    - Procedure: Inject vasopressin → measure rise in blood pressure via carotid artery or tail-cuff method.
2. In vitro: Isolated rat aorta or rabbit blood vessel
    - Observe vascular contraction response.

Remarks:

- Standard vasopressin used for comparison.
- Highly sensitive; avoid excessive handling of animals.

ACTH (Adrenocorticotropic Hormone)

Principle:

ACTH stimulates adrenal cortex → increases corticosterone/cortisol synthesis.

Bioassay: Rat adrenal cortical assay

- Procedure: Inject ACTH into adrenalectomized or intact rats → measure adrenal weight increase or corticosteroid level in plasma/urine.
- Alternative: Radioimmunoassay (modern method) for cortisol.

Remarks:

- Adrenal weight gain is proportional to ACTH dose.
- Classical method is labor-intensive; replaced by immunoassays in modern labs.

D-Tubocurarine

Principle:

D-tubocurarine is a non-depolarizing neuromuscular blocker → inhibits nerve-mediated skeletal muscle contraction.

Bioassay: In vitro frog rectus abdominis or rat phrenic nerve-diaphragm preparation

- Procedure: Apply d-tubocurarine → stimulate nerve electrically → measure decrease in muscle contraction.
- Alternative: Chick biventer cervicis muscle.

Remarks:

- Sensitive to temperature and ionic composition.
- Reversible by acetylcholine or neostigmine in some setups.

Digitalis (Cardiac Glycosides)

Principle:

Digitalis increases force of contraction (positive inotropy) in cardiac muscle.

Bioassays:

1. In vitro: Isolated frog heart or mammalian atrium
    - Measure force and rate of contraction on kymograph after applying digitalis.
2. In vivo: Dog or rabbit
    - Monitor ECG changes or cardiac output.

Remarks:

- Narrow therapeutic index; careful dosing required.
- Modern labs use immunoassay for serum digitalis levels.

Histamine

Principle:

Histamine produces contraction of smooth muscle (gut, bronchus) and vasodilation in blood vessels.

Bioassays:

1. In vitro:
    - Guinea pig ileum: Histamine → contraction of ileal smooth muscle (H1 receptor mediated)
    - Rat fundus: Contraction via H2 receptor
2. In vivo:
    - Measure hypotensive effect or gastric acid secretion.

Remarks:

- Responses blocked by antihistamines (H1/H2).
- Tissue preparation must be fresh; pH and Ca^{2+} critical.

5-HT (Serotonin)

Principle:

5-HT acts on smooth muscles, platelets, and CNS → vasoconstriction, gut contraction.

Bioassays:

1. In vitro: Isolated tissues
    - Guinea pig ileum → contraction via 5-HT2 receptors
    - Rabbit aorta → vasoconstriction
2. In vivo:
    - Measure blood pressure rise or gut motility in rats.

Remarks:

- Effects blocked by specific 5-HT antagonists
- Dose-response curves used to determine potency