Laboratory Manual

PHARMACOLOGY-II

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About MLRIP

VISION

To be an educational Institute of par excellence and produce competent pharmacy professionals to serve the community through research and the ever-increasing needs of Industry.

MISSION

1. Imparting quality education and innovative research for various career opportunities.
2. Creating conducive academic environment to produce competent pharmacy professionals.
3. Indoctrination of students adorned with high human values and make them aware of their responsibility as health care professionals.

Program Educational Objectives

PEO 1: To produce graduates with sound theoretical knowledge and technical skills required for their career opportunities in various domains.

PEO 2: To incite the students towards research and to address the challenges with their innovative contributions for the benefit of the mankind.

PEO 3: To instill the essence of professionalism, ethical commitment to become a health care professional with sound integrity and adherence to the core human values in the service of the society.

PROGRAM OUTCOMES

1. **Pharmacy Knowledge:** Possess knowledge and comprehension of the core and basic knowledge associated with the profession of pharmacy, including biomedical sciences; pharmaceutical sciences; behavioral, social, and administrative pharmacy sciences; and manufacturing practices.
2. **Planning Abilities:** Demonstrate effective planning abilities including time management, resource management, delegation skills and organizational skills. Develop and implement plans and organize work to meet deadlines.
3. **Problem analysis:** Utilize the principles of scientific enquiry, thinking analytically, clearly and critically, while solving problems and making decisions during daily practice. Find, analyze, evaluate and apply information systematically and shall make defensible decisions.
4. **Modern tool usage:** Learn, select, and apply appropriate methods and procedures, resources, and modern pharmacy-related computing tools with an understanding of the limitations.
5. **Leadership skills:** Understand and consider the human reaction to change, motivation issues, leadership and team-building when planning changes required for fulfillment of practice, professional and societal responsibilities. Assume participatory roles as responsible citizens or leadership roles when appropriate to facilitate improvement in health and well-being.
6. **Professional Identity:** Understand, analyze and communicate the value of their professional roles in society (e.g. health care professionals, promoters of health, educators, managers, employers, employees).
7. **Pharmaceutical Ethics:** Honour personal values and apply ethical principles in professional and social contexts. Demonstrate behavior that recognizes cultural and personal variability in values, communication and lifestyles. Use ethical frameworks; apply ethical principles while making decisions and take responsibility for the outcomes associated with the decisions.
8. **Communication:** Communicate effectively with the pharmacy community and with society at large, such as, being able to comprehend and write effective reports, make effective presentations and documentation, and give and receive clear instructions.
9. **The Pharmacist and society:** Apply reasoning informed by the contextual knowledge to assess societal, health, safety and legal issues and the consequent responsibilities relevant to the professional pharmacy practice.
10. **Environment and sustainability:** Understand the impact of the professional pharmacy solutions in societal and environmental contexts, and demonstrate the knowledge of, and need for sustainable development.
11. **Life-long learning:** Recognize the need for and have the preparation and ability to engage in independent and life-long learning in the broadest context of technological change. Self-assess and use feedback effectively from others to identify learning needs and to satisfy these needs on an ongoing basis.

LABORATORY ORIENTATION

The laboratory portion of this course is designed to study the different laboratory animals, their applications and screening of different pharmacological agents thoroughly than it is presented in lecture. Core learning will come from practical and tissue studies. This method of 'hands on' learning should also enhance and strengthen the knowledge you gain in lectures.

At times you will be working individually, in pairs or in groups of three or four. Each lab period is loosely structured to begin with a short introduction to the exercise that highlights the activities of the day, what materials are available for use and any changes in procedures. After that you will work independently to learn the material.

There is never enough time in lab to go over each and every item that you are assigned. The lab is a designated a time when you have access to materials that you will not have available during home study time. Some of the information assigned in lab you can learn at home, particularly animals, instruments, method, procedure, application, mechanism of action of drugs etc.

GENERAL OBJECTIVES OF THE COURSE

At the end of the practical training in general, and experimental pharmacology the learner shall be able to:

1. List the various dosage forms and enumerate their advantages and disadvantages.
2. Advise patients about the proper use of medication devices, storage of medicines etc.
3. Retrieve drug information from appropriate sources.
4. Appreciate the role of good laboratory practice in promotion of rational diagnostics, therapy, and experimentation.
5. Realize the cardinal role of ethics in experimentation.
6. Order monitoring of drug levels where indicated and take appropriate remedial measures.
7. Prescribe rationally and in an individualized pattern.
8. Plan and carry out experiments to demonstrate the effect of drugs in experimental animals and isolated tissues.
9. Critically appraise drug advertisements.
10. Apply fundamental statistical tests to experimental data and interpret results.

EXPERIMENTAL NO- 01

Object: Basic principle of Bioassay.

Introduction:-

Bioassay is defined as estimation or determination of concentration or potency of physical, chemical or biological agents by means of measuring and comparing the magnitude of the response of the test with that of standard over a suitable biological system under standard set of conditions. An assay is a form of biological experiment, but the interest lies in comparing the potencies of treatments on an agreed scale, instead of in comparing the magnitude of effects of different treatments. Biological assays or biological standardizations or simply bioassays are methods used for estimation of the potency of substances by observing their pharmacological effects on living animals (*in vivo*) or isolated tissues (*in vitro*) and comparing the effect of these substances of unknown potency to the effect of a standard.

Principle of Bioassay:-

Active principle to be assayed should show the same measured response in all animal species. Bioassay involves the comparison of the main pharmacological response of the unknown preparation with that of the standard. The method selected should be reliable, sensitive, and reproducible and should minimize errors due to biological variation and methodology. The degree of pharmacological response produced should be reproducible under identical conditions. The reference standard and test sample should have same pharmacological effect and mode of action, so that their DRC curve run parallel and their potency ratio can be calculated. Activity assayed should be the activity of interest. Individual variations must be minimized/accounted for. Bioassay might measure a diff aspect of the same substance compared to chemical assay. The test solution and standard should be compared for their established pharmacological effect using a specified pharmacological technique.

Types of Bioassays

There are three main types of bioassays (other than qualitative assays):-

1. Direct Assays
2. Indirect Assays based upon quantitative responses

3. Indirect Assays based upon Quantal responses ("all or none")

- **Direct Assay:-** Doses of the standard and test preparations are sufficient to produce a specified response, and can be directly measured.
- **Indirect Assay:-** In indirect bio-assays the relationship between the dose and response of each preparation is first ascertained. Then the dose corresponding to a given response is obtained from the relation for each preparation separately.
- **Quantal Assay:-** This response is in the form of "all or none" means no response or maximum response. These can be biossayed by end point method. Predetermined response is measured which is produced by threshold effect. Quantal Responses are population response based on an all-or-nothing (0 or 1 – presence or absence) response such as death.

Concentration of unknown = Dose of the standard × Concentration of standard/ Dose of test

- **Graded Assay:-** It is proportional to the dose and response may lie between no response and maximum response. Graded Responses can be any type of measured responses in isolated tissues in particular, but also in whole animals. Such responses are infinitely graded and there are a large number of them.

Examples – contractions of muscle, blood pressure, blood sugar concentrations, etc.

- **Matching Method:-** In this type of assay the test substance and the standard are applied and the responses obtained are matched by a trial and error process until they produce equal effects.
- **Bracketing Method:-** Bracketing bioassay is performed by selecting two standard doses, which will give a close bracket on either side of the response produced by the unknown. The working dose of standard is first determined in the sensitive part of dose-response curve, that is, a dose that will approximately produce 50% of the maximal concentration.
- **Interpolation Method:-** This is a simplest form of graded response assay and involves no statistical data and many calculations. In this assay the dose response

curve is fist obtained from different doses of standard ach solution. The concentration of unknown is then read from the standard graph. Interpolation method of bioassay is less time consuming and yet reliable compare to matching type of bioassay.

- **Three Point Bioassay:-** In three point bioassay, the DRC of standard & test samples is first obtained from the responses due to graded doses. From the DRC of standard, two standard doses are selected in such a way that they have produced 25% & 50% of the maximal response respectively & are designated as S1 & S2. The responses of these doses lie on the steepest & straightest part (linear) of the curve. From the DRC of test sample one test is selected such that it gives a response which lies in between the two standard responses that is it gives a greater response than S1 & a smaller response than S2 & is designated as T. After selecting the standard & test doses, the bioassay is performed by recording the standard & test responses in randomized fashion as per Latin square design. The pattern of addition of doses is S1, S2, T; S2, T, S1 & T, S1, S2 in 3 successive cycles. The mean values of height of contraction for all the 3 doses are calculated and are used in plotting the graph so as to estimate the potency of the test sample. The precision and reliability of this method is much better than matching and bracketing methods of bioassay & the sensitivity of the isolated tissue preparation is assessed prior to testing the unknown sample.
- **Four Point Bioassay:-** The classic 2X2 parallel assay involves being able to measure parallelism where drugs acting through the same mechanism are expected to produce parallel dose-response curves.

EXPERIMENTAL NO- 02

Object: To study the dose response curve of ach using rati leum

REQUIREMENTS:

Animal: Rat (of either sex weighing between 200-250g.)

Drugs: Acetylcholine (1 µg/mL, 10 µg/mL, 100 µg/mL)

Apparatus: Reservoir, Tubing, isolated organ bath, organ tube heating coil, Thermostat, Isotonic frontal writhing lever, Recording drum, Aeration tube cum tissue holder, Haemostatic forceps, sketch pen tip, ink etc.

EXPERIMENTAL CONDITION:

Physiological Salt Solution	: Tyrode
Temperature	: 37 ± 1^{o} C
Basal Tension on Lever	: 500 mg
Contact time	: 30 sec.
Aeration (95% O_2 and 5% CO_2)	: Carbogen
Magnification of the response	: 10 times
Drug	: Acetylcholine Chloride (1, 10 or 100 µg/mL)
Molecular weight of drug	: 181.78

PRINCIPLE:

Acetylcholine produces a dose dependent concentration of rat ileum smooth muscle. First taken the two equipotent response of same dose and then taken the graded response.

THEORY:

Graded Dose Response Relationship Curve of Acetylcholine on Frog Rectus Muscle:

- Single biological unit, either a single animal or an isolated tissue is used.
- It depends upon an observation that graded increase (in geometric proportion) in the dose of drug gives proportional rise in the magnitude of biological response.

- Actually, beyond a specific dose level, biological response increases in proportion to the increase in dose. This dose level is known as 'Threshold dose'.
- Such proportional rise in biological response occurs only up to a dose level known as 'Ceiling dose', beyond which a steady biological response is achieved even after increasing the doses.
- Shape of Graded DRC, when plotted as 'dose Vs Response' is a 'Parabola'

- Shape of 'Log Dose Vs Response' curve is a 'Sigmoid' line or is having 'S' like shape.

PROCEDURE:

- The assembly is set up and the arrangements are made for the above mentioned condition.
- A rat fasted over night was anaesthetized by chloroform and sacrificed by as per CPCSEA recommended guidelines.
- The abdominal cavity was quickly opened through a midline incision, leum is separated and mounted in the organ bath.
- One end of the ileum is tied to the aeration tube and the other is connected to the isolated frontal writing lever.
- The ileum is allowed to stabilize for half an hour. During this period the PSS is changed after every ten min. Once the tissue is stabilize, raded doses of Ach are added to at defined time period of interval for obtain contractile responses.
- 00 sec: Start the drum and record a base line for 30 sec.
- 30 sec: Add the first dose of drug in organ bath and take the response for another 30sec.
- 60: Stop the drum and give wash until the tip of lever rich to baseline.
- Continue above procedure for next doses.
- Measure the height of concentration at different doses of Ach.
- Tabulate the observations into three columns as Dose of Ach, Height of concentration (in mm) and % response.

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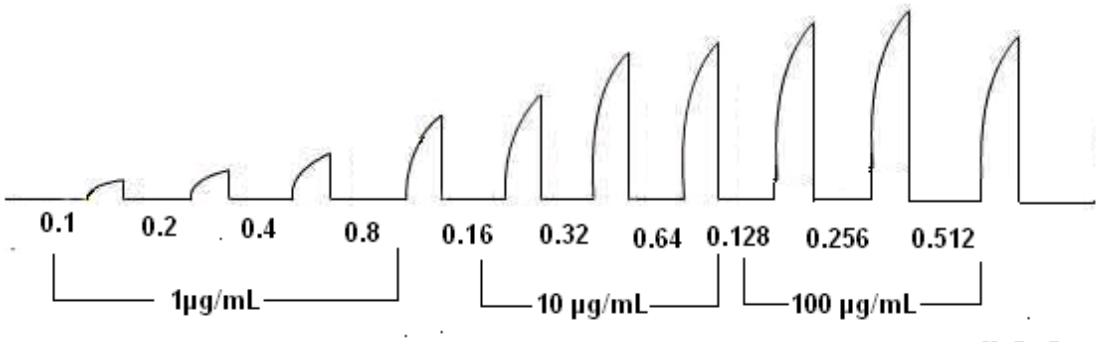

OBSERVATION TABLE:

Sr. No	Drug Name	Conc. of drug	Dose of drug in mL	Response in mm	% Response
1.	Ach	1 µg/mL	0.1	2	20
2.			0.2	5	25
3.			0.4	7	35
4.			0.8	9	45
5.		10 µg/mL	0.16	12	60
6.			0.32	14	70
7.			0.64	16	80
8.		100 µg/mL	0.128	17	85
9.			0.256	20	100

EXPERIMENTAL NO- 03

Object– To perform and determine the strength of an unknown sample by three point bioassay using isolated organ preparation.

References – Kulkarni S.K., Handbook of experimental pharmacology, Vallabh prakashan, first edition 2009, page no.92-94

Requirements –

Drug - Histamine stock solution

Physiological solution – Tyrod solution

Procedure –

1. The guinea pig is scarified by a blow on the head and carotid blending.
2. Cut open the abdomen and lift the caeccum to trace the ileo-caecum junction cut and remove a few centimeters long of ileum portion and immediately place it in the watch glass containing tyrode solution.
3. Take one piece of ileum of 2-3 cm long and tie the thread to top and bottom ends without closing the lumen ,mount the tissue in the organ bath containing Tyrode solution maintained at 32-35^{0}c and bubbled with 0.2 or air.A tension of 0.5gis applied and the tissue is allowed to equilibrate for 30 min. before adding drug to the organ bath.
4. Record concentration dependent response due to histamine using frontal writing lever.\contact time of 30 sec. and 5 min. proper recording of the response.
5. Record at least 4 concentration dependent response curve due to histamine.
6. Select two doses standard say S_1 and S_2 which produce sub maximal response.
7. Select a dose of the test sample t, which is likely to produce a response less than the response produced by S_2 dose of the standard as shown below.

8. Measure the of concentrated produced by S_1 dose as $S_{1.}$
9. Measure the height of concentration produced by S_2 dose.
10. Measure the height of concentration produced by S_2 dose.
11. Measure the height of concentration produced by t_1 dose as $t_{1.}$.
12. Calculate the potency of the test solution.

Potency and test solution:

$$\frac{S_1}{T_1} \text{ X antilog } \frac{T_1 - S_1}{S_2 - S_1} \text{ X log } \frac{S_2}{S_1}$$

Results:-

The method for finding the strength of an unknown sample of Histamine by three point bioassay using Guinea pig ileum preparation had been demonstrated.

EXPERIMENT NO. – 04

Object – To perform and determine the strength of an unknown sample of histamine by four point bioassay using isolated organ preparation.

Reference – Kulkarni S.K. Handbook of experimental pharmacology, vallabh prakashan first edition 1987 reprint 2009, page no.92 to 94

Requierment –

Animal – Guinea pig (400-600gm overnight fasted)

Drugs – Histamine stock solution

Physiological solution –Tyrode

Procedure –

1. The guinea pig is sacrifieced by a blow on the head and carodid bleeding.
2. Cut open the abdomen and till the caeccum to trace the ilium caecal junction. Cut and remove a few centimeter long of the real portion and immediately place it in the watch glass containing tyrode solution trim the nesentry and with gentle care olean the contents of the ileum by pushing the tyrode solution into the lumen of the ileum. Cut the ileum into small segments of 2-3 c.m. longs.
3. Take one piece of ileum of 2-3 c.m. long and tie the thread to top and the bottom ends without closing the lumen and mount the tissue is allowed to equilibrate for 30 min. before adding drugs to the organ bath.
4. Record concentration dependent response due to histamine using frontal writing lever, contact time of 30sec. and 5 min. time cycle are kept for proper recording of the responses.
5. Record at least 4 concentration dependent response due to histamine.
6. Record graded response with the standard solution histamine until peak effect is observed.
7. Select two concentration (A,B) of the standard drug, elicting sub-maximal

drug responses(S_1,S_2) andbearing a dose ratio 1:2 preferentially.

8. Select two suitable volume of the test solution by trail and error method in such a waythat the response(T_1) due to the lower dose of the test (C) lies preferentially between S_1 and S_2 . the higher volume of the test solution selected would be D such that the dose ratio B/A = D/C all the four responses (S_1,S_2 , T_1, T_2) due to the doses thus selected (A,B,C,D) must lie on the linear part of standard curve.
9. Standardise the tissue with the concentration A.
10. Record four sets of responses due to A,B,C,and D,adding them to organ bath in a random fusion.
11. Label and fix the tracing.
12. Measure various responses to calculate the mean of each (S_1,S_2 , T_1, T_2)
13. Calculate the potency ratio(M) using following formula:-

Potency ratio = x_1/ y_1 X antilog

$$\frac{T2-S2+T1-S1}{T2-T1+S2-S1} \; X \; log \frac{X2}{X1} = M$$

Where, X1 = Lower volume of the standard drug (A)

X2 = Higher volume of the standard drug (B)

Y1 = Lower volume of test solution (C)

Mean S and T = the mean responces

14. Determine the strngth of the unknown solution of histamine using the concenteration of the standard drug and potency ratio (M).

    Report – the method for finding the strength of an unknown sample of histamine by four point bioassay using guinea pig ileum preparation has been demonstrated.

Results:-

The method for finding the strength of an unknown sample of Histamine by four point bioassay using Guinea pig ileum preparation had been demonstrated.

EXPERIMENT NO.-5

Object: - To record the concentration response curve of Oxytocin using rat uterus preparation.

Requirement:-

Animal- Female rat (120-150gm)

Drugs Oxytocin, Stilbesterol, Dejalon solution.

Procedure:-

1. Examine the vaginal snear under microscope to know about the proper stage of oestrus cycle, if the rat is not in Frank oestrus inject 0.1 mg/kg of stilbesterol and wait for 24 hours.(Vaginal smear is prepared by taking a drop of the slide glass)
2. Cut open the pelvic region and expose both the horn and uterus separate them gently from the surrounding fatty material and transfer them to a dish containing DeJalon solution. When the rat is in oestrus generally the uterus is fleshy and pink in colour.
3. Two separate pieces (2-3cm long) of uterine preparation can be made.
4. Mount the uterine preparation in the organ bath containing DeJalon solution at 30-32^{0}C.Apply a tension of 0.5gm and allow the tissue to equilibrate for 30 minutes.
5. Record contraction due to different coenteration of Oxytocin using frontal writing lever.Contact time of 30 seconds and 3 minutes time cycle is used for proper recording of the responses.
6. Label and fix the tracing and draw the concenteration-response curve.

Results:-

The CRC of Oxytocin using rat uterus has been recorded.

EXPERIMENT NO.-6

Object: - To study the anti-secretory and ulcer protective effect of cimetidine in pylorus-ligated rats.

Reference: - (1) Gupta S.K., Drugs screening methods, first edition,2004, Jaypee Brothers publication, Page no.- 301-307.

(2) Indian Drugs, Volume 48, issue no.- 03, march 2011,page no.- 31-32.

Requirement:-

Animal-	Wistar albino rat (150-200gm)
Drugs	Cimetidine (Dose 10 mg/kg, ip)
	Topfer's Reagent

Equipment:

Dissecting microscope, surgical equipment's

Procedure:

1. Anaesthetize the overnight fasted animal.
2. Give an incision of 1 cm long in the abdomen just ellow the sternum.
3. Expose the stomach. Pass a thread around the pyloric sphincter and apply a tight knot.
4. To another rat inject cimetidine. After 15 min perform pyloric ligation as described.
5. Give a small cut to the pyloric region just above the knot and collect the contents of the stomach in a centrifuge tube.
6. Open the stomach along the greater curvature and wash it slowly under the running tap water.

   Score the ulcer as bellow:

   0= Normal colored stomach

   0.5= Red colouration

   1= Spot ulcers

   1.5= Haemorrhagic sreaks

2= ulcer ≥ 3 but ≤ 5

3= ulcers > 5

Mean ulcer score for each animal is expressed as ulcer index.

OBSRVATION TABLE

Sr. No	**Treatment**	**Dose (mg/kg)**	**Ulcer Index**

Report: