JHARKHAND
Rai University
UGC RECOGNISED UNIVERSITY
ACCREDITED BY NAAC

PRACTICAL LAB MANUAL

PHARMACOLOGY

D Pharm IInd Year

Lists of Experiment

1. Introduction to experimental pharmacology and pharmacy. Sources of drugs
2. Study of action of drugs on the rabbit's eye
3. Study of effect of drugs on ciliary movement of frog's oesophagus
4. Study of effect of drugs on frog's rectus muscle preparation
5. Effect of cardiac stimulants and depressants on perfused frog's heart
6. Effect of drugs on dog's blood pressure and respiration - computer assisted learning (CAL) method
7. Evaluation of analgesics by chemical method
8. Effect of saline purgative on frog intestine and the use of Oral Rehydration Solution.
9. Preparation of solution for test dose of penicillin
10. Study of action of antidepressants on mice
11. Study of anorectic and locomotor activity of amphetamin and fenfluramine.

EXERCISE NO.1

OBJECTIVES: INTRODUCTION TO EXPERIMENTAL PHARMACOLOGY AND PHARMACY SOURCES OF DRUGS

1. List the various sources of some common drugs and identify them
2. Define the various terms relating to the science of pharmacology such as pharmacy, toxicology, therapeutics, clinical pharmacology, ethnopharmacology etc.,
3. Be familiar with the layout of the dept. of pharmacology, the various laboratories, animal room and staff and faculty.
4. Understand what is expected of them during the practical classes, the method of internal assessment, allocation of marks etc.,

EXERCISE NO. 2

OBJECTIVES - STUDY OF THE ACTION OF DRUGS ON THE RABBIT'S EYE

At the end of the practical class the student shall be able to:

1. Instill drugs carefully into the rabbit's eye by the pouch method without injuring the cornea.
2. Study the effects of drugs on the rabbit's eye.
3. Record, analyze and interpret the observations obtained during the experiment.

Animals: Rabbits
Apparatus: Droppers, measuring scale, torch, cotton wool, calculator (optional) **Drugs & solutions:**

1. Saline
2. Eserine salicylate 0.5%
3. Atropine sulphate 1.0%
4. Phenylephrine 2.0%
5. Lignocaine 1.0%

PROCEDURE

Place the rabbit (No.1) on the table. Measure the diameter of both the pupils with the help of a scale. Observe the condition of the conjunctiva (congested or not) and elicit the corneal and light reflexes. Record your findings. In the left eye put one drop of saline and in the right eye one drop of eserine. Use the pouch method for instilling the drops. After adding the drops, the medial canthus should be pressed for 10 seconds. Record the following parameters at one minute, 5minutes and ten minutes after instilling the drug and saline. Parameters to be measured:

1. Diameter of the pupil
2. Light reflex
3. Corneal reflex

Record your observations in a tabular form. Repeat the same procedure for atropine, phenylephrine and lignocaine on separate rabbits (Nos 2,3 & 4).

Presentation of data and analyses:

Pool the data from other groups and formulate appropriate table(s) to display the data. Analyze the data using appropriate statistical test(s) and draw conclusions. Make sure that table(s) is/are complete in all respects.

EXERCISE NO. 3

OBJECTIVES - STUDY OF EFFECT OF DRUGS ON CILIARY MOVEMENT OF FROG'S OESOPHAGUS

1. Demonstrate the action of drugs on ciliary movement of frog oesophagus.
2. Record and interpret correctly the observations obtained.
3. List uses of cholinergic and anticholinergic drugs and explain the basis for their use in eachcondition.

Animals: Frog

Drugs and solutions:

Acetylcholine 100µg/ml
Physostigmine 100µg/ml
Atropine 1µg/ml
Frog's Ringer

Apparatus: A pair of scissors, forceps, poppy seeds, cotton, droppers, frog board, stop-watch

PROCEDURE

Pith a frog. Slit open the oesophagus from the buccal cavity to the stomach. Wipe the blood gently using a cotton swab dipped in Frog's Ringer solution, proceeding from cephalic to caudal end. Moisten the surface with Ringer solution. Place two pins at a distance of 2-3 cm. Place one seed on the groove near the pin at cephalic end. Start the stopwatch and observe the time taken for the seed to reach the pin at the caudal end. Take 2 such readings and calculate the average. Repeat the experiment using acetylcholine, physostigmine and atropine. Take control readings with Frog's Ringer between the drugs.

Presentation of data and analyses:

Pool the data from other groups and formulate appropriate table(s) to display the data. Analyze the data using appropriate statistical test(s) to find out whether the given drugs produce significant effects in comparison with Ringer. Draw conclusions and record them. Make sure that table(s) is/are complete in all respects.

EXERCISE NO. 4

OBJECTIVES - STUDY OF EFFECT OF DRUGS ON FROG'S RECTUS MUSCLE PREPARATION

At the end of the practical class the student shall be able to:

1. Record the dose-response curve of acetyl choline on isolated frog's rectus muscle and demonstrate the effect of eserine and d-tubocurarine on it.
2. Interpret the observations and explain the basis for the same.
3. Understand the rationale for the use of skeletal muscle relaxants in surgery and the pharmacological basis for reversal with neostigmine.

Animal: Frog

Apparatus: Isolated organ bath, student's physiograph / recording drum with smoked paper, writing lever, aerator, syringes and needles

Drugs and Solutions:

Acetylcholine	100□g/ml
Eserine	1mg/ml
Tubocurarine	100□g/ml
Frog's Ringer	

Preparation: Isolated frog rectus muscle is mounted in an organ bath filled with aerated frog's Ringer solution. The muscle is allowed to relax for 45 mins under 5 gm tension and washed intermittently.

PROCEDURE

1. Graded response with Acetylcholine (Ach): Start the experiment by giving a test dose of 10 □g of Ach. Increase the dose if required and check the reproducibility of the response by repeating the same dose 2-3 times. Increase the dose by doubling each time till maximal response is reached. For each response start the stopwatch soon after the drug is added to the bath. After 90 secs. stop the drum, drain the bath and wait for 4 mins. Choose a working dose of Ach which produces 40-60% of maximal response and obtain 1 response with this dose.

2. Add Eserine 100 □g to the bath and note its effect for 90 secs. Stop the drum and wait for 10 mins. Add the working dose of Ach and note the response. Wash the tissue 3 times and wait for 5 mins.

3. Repeat step 2 with d-tubocurarine (50□g) and note its effect on the Ach response.

4. Measure all the heights of contractions and plot a dose response as well as a log dose response curve. Also mark the effects of eserine and tubocurarine on Ach response in the graph paper.

EXERCISE NO. 5

OBJECTIVES - EFFECT OF CARDIAC STIMULANTS & DEPRESSANTS ON PERFUSED FROG'S HEART

At the end of the practical class the student shall be able to:

1. Record the contractions of isolated frog's heart on a kymograph and demonstrate the effect of drugs on it.
2. Interpret the observations and explain the basis for the same.
3. List the cardiac stimulants & depressants and understand the rationale for their use in therapy.

Animal: Frog

Drugs and solutions:

1. Adrenaline HCl 10 □g/ml
2. Noradrenaline 10 □g/ml
3. Isoprenaline 10 □g/ml
4. Calcium chloride 10 mg/ml
5. Propranolol HCl 1 mg/ml
6. Acetyl choline 10 □g/ml 7.Potassium chloride 10 mg/ml
8. Atropine sulphate 100 □g/ml

Frog's Ringer

Apparatus: Frog heart perfusion apparatus, Student's physiograph / Starling's heart lever, smoked drum, syringes, needles

Preparation:

Frog is pithed, dissected, the heart is removed and perfused through the sinus venosus. Insert a curved needle in the apex of the heart and attach it to a Starling's heart lever. Record the contractions.

PROCEDURE

1. Note the normal heart rate, by counting each upstroke (systole) and downstroke (diastole) of the moving drum together as one beat for 1 minute, force of contraction (by measuring the amplitude or height of the contraction from the baseline with a scale), tone (by observing shift in the baseline) and the cardiac rhythm (by observing any irregularity in the contractions).

2. Inject 0.2 ml of drugs 1-4 in succession (cardiac stimulants) in the tube through which the heart is being perfused and record the responses. A control reading (without addition of any drug) should be taken before and after each drug response. All the parameters mentioned above should be recorded during the control and drug responses respectively. The heart rate, drug name and the

dose should be mentioned in the recording during the control and drug responses. The next drug response should be recorded only after the heart rate has returned to the approximate original value. In case the heart stops because of systolic or diastolic arrest produced by a cardiac depressant the drum should be stopped and re-started only when the heart is contracting. In case adequate response is not observed use a higher dose.

3. Inject 0.2ml of propranolol (depressant) and note its response. Stop the drum for 5 minutes. After 5 minutes inject adrenaline (same dose as injected previously) and note whether its effect is adequately blocked. In case sufficient blockade is not obtained repeat the procedure with 0.4ml propranolol and also see that the 5 minutes duration is adhered to.

4. Inject Calcium chloride immediately after adrenaline effect has been blocked, and note whether its effect has been blocked or not. In case the typical increase in time and /or systolic arrest is not observed use higher dose.

5. Inject 0.2 ml of drugs 6 & 7 in succession (cardiac depressants) i.e acetylcholine and potassium chloride after taking control readings in between drug responses. Note also the condition of the heart during systole arrest (contracted) and diastolic (dilated).

6. Inject 0.2 ml of Atropine and note its response. Normally no response is seen because it is an in vitro preparation and moreover atropine has no intrinsic activity of its own. Stop the drum and wait for 5 minutes, inject acetylcholine (same dose as given earlier) and note whether effect is completely blocked. In case sufficient blockade is not obtained, repeat the same procedure with 0.4ml of atropine and see that the 5 minutes duration is adhered to.

7. Finally inject potassium chloride after the effect of acetylcholine has been blocked by atropine and note whether the effect is blocked. There should be no blockade of KCl effect.

8. Tabulate your observations.

9. Pool the data from all the other groups and apply a suitable statistical test. Write down the conclusions and inferences.

EXERCISE NO. 6

OBJECTIVES - EFFECT OF DRUGS ON DOG'S BLOOD PRESSURE AND RESPIRATION - COMPUTER ASSISTED LEARNING (CAL) METHOD

At the end of the practical class the student shall be able to:

1. Explain the effect of drugs acting on the autonomic nervous system on blood pressure, heart rate and respiratory rate.

2. Identify an unknown drug using these three parameters and its interaction with other known drugs

Note: This experiment is demonstrated using Ex Pharm - a computer simulation software programme .

EXERCISE NO. 7

OBJECTIVES - STUDY OF ANALGESICS BY CHEMICAL METHOD

At the end of the practical class the student shall be able to:

1. Calculate and measure the exact quantity of drug to be injected to the animal.
2. Administer the drugs by the subcutaneous and intraperitoneal routes to mice.
3. Identify and record experimentally induced writhing in mice.
4. Tabulate observations and draw suitable inferences from the experiment.

Chemical Method: Acetic acid induced writhing test

Animals: 2 Mice

Apparatus: Syringe (with 100 divisions) 26g needle

Drug & Solutions:

Morphine sulphate	2mg/ml
Acetic acid	0.6%
Saline	

PROCEDURE

Weigh 2 mice (in mg) and number them. Pretreat one mouse with morphine 3mg/Kg sc and the other with saline (0.02ml) sc. Note time of injection. 30 minutes after injection of drug/saline, inject 0.2ml of 0.6% acetic acid ip to each mouse with 26g needle. Observe the no. of writhings (stretching syndrome) occurring in the next 15 minutes. Record each observation and then total. One writhe is taken as the complete movement from side to side (both sides). Observe only one mouse at a time. Number of writhings to be noted for 15 minutes from the time of administration of acetic acid. Tabulate your observations. Pool the data from other groups, tabulate the data, use appropriate statistical tests and draw inferences based on the statistical analysis.

Check calculated volume of drug to be injected. Check volumes in syringes prior to injection. Restrain the animal for inject. Timings of injections should be noted. Only one animal to be observed at a time.

1. Acetic acid should be freshly prepared for each class.
2. Make sure all groups pool data calculate mean and SD and apply unpaired Student's t test.
3. The experiment can be completed in 90 minutes. This can be followed by a small group discussion on evaluation of analgesics in humans.

EXERCISE NO. 8

OBJECTIVES - EFFECT OF SALINE PURGATIVE ON FROG INTESTINE AND THE USE OF ORAL REHYDRATION SOLUTION

At the end of the practical class the student shall be able to:

1. Dissect and demonstrate the effect of saline purgatives on frog intestine.
2. Record observations and make suitable inferences based on them.
3. List the components of ORS and explain the function of each component.
4. Instruct patients to prepare and use ORS in the home.
5. Understand the rationale behind the use of ORS and appreciate the danger of using a ORS formula which does not conform to these standards.

The batch is divided into 4-5 small groups and the animal experiment is conducted as described below. After the experiment (one hour) each group is asked to prepare Oral Rehydration Solution (ORS). Then all groups are collapsed to make the batch and a role play can be done to bring out the communication aspect of instructing a patient/mother to prepare and use ORS at home. A focussed group discussion will follow on problems of using solutions with high glucose content.

Animals: Frog

Apparatus: Frog's board, dissecting instruments, pithing needle, needle with thread, tuberculin syringe with needle.

Drugs and solutions:

Magnesium Sulphate (Hypertonic)	27%
Saline (Hypotonic)	0.9% made upto 0.45%
Frog's Ringer (Isotonic)	

PROCEDURE

Pith a frog. Expose the abdominal cavity. Trace the small intestine and make three compartments by tying threads at equal distance. Secure the threads tightly so that no fluid can seep through from one compartment to the other. In the first compartment, inject 0.2ml of hypotonic saline, 0.2ml of magnesium sulphate in the second compartment, and 0.2ml of Frog's Ringer in the third compartment. Wait for 20 minutes and record the observation.

OBSERVATION

First Compartment: 0.2ml of saline (hypotonic) has been injected. The ileal loop was shrunken after 20 minutes.

Second Compartment: 0.2ml of Magnesium sulphate was injected. The ileal loop was found to be swollen after 20 minutes.

Third Compartment: 0.2ml of frog's ringer has been injected. After 20 minutes no change was seen in the size of the compartment.

INFERENCE

1. Hypotonic saline causes movement of fluid from the lumen into circulation by osmosis.
2. Magnesium sulphate by osmosis, causes movement of fluid from the cells into the lumen.
3. Ringer is Isotonic with frog's blood and there is no flow of fluid across the intestinal membrane.

Group task on ORS:

Demonstrate how to prepare ORS using things which are readily available in the house.

(1 litre and 200ml) Role play

Enact a role play to bring out the salient features regarding the preparation and use of ORS in diarrhoea. (One student is the doctor and one is the mother of the child with diarrhoea).

1. 2 teaspoonfuls of sugar and a pinch of salt is added to a glass (200ml) of boiled and cooled water. The ORS solution should be used within 24 Hrs. Left over solution should be discarded. The solution should be given to the patient (as much as he/she can drink), after every stool. Do not stop breast feeding or feeding the child during episodes of diarrhoea.

EXERCISE NO. 9

OBJECTIVES - PREPARATION OF SOLUTION FOR TEST DOSE OF PENICILLIN

At the end of the practical class the student shall be able to:

1. Dispense a 5 ml solution of sodium penicillin G for intradermal sensitivity testing.
2. Take adequate aseptic precautions during the preparation of the solution.
3. List the sign and symptoms of the anaphylactic reaction to penicillin and the measures to be taken to treat such a reaction

A brief introduction on the signs & symptoms of anaphylactic shock to penicillin and its treatment is given. (15 minutes) The batch is divided into small groups of 5 – 6 students and they are asked to prepare the solution as a group. The students are asked to dispense a 5 ml solution of sodium penicillin G for intradermal sensitivity testing. (1 hour). Small group discussions on aseptic precautions 10-15 minutes) can be conducted when the group completes its task.

Task: Prepare and dispense a 5 ml solution of sodium penicillin G for intradermal testing.
from the given stock solution of 10 lacs vial.

Composition:
Sodium penicillin G 100 Units Water
for injection to make 1 ml

Procedure:
Penicillin G sodium 10,00,000 units
Water for injection 100 ml
Mix under sterile conditions. Send such 5 ml.
Inject 0.02 ml intradermally on the forearm and observe the reaction for 30 minutes.

To a vial containing 10,00,000 units/vial of Sodium Penicillin G add 5 ml of water for injection and shake to dissolve and obtain 2,00,000 units/ml. Take 0.1ml of this solution in a syringe and transfer it to an autoclaved 30 ml vial. Dilute to obtain 20ml by adding 19.9 ml of water for injection with the help of a sterile 20ml syringe. This solution now contains 1000 units/ml. Transfer 0.5 ml of this solution to another small sterile vial and dilute to 5 ml by adding 4.5 ml water for injection.

Label
Penicillin G 100 units/ml solution for sensitivity testing

1. Bring 1 ml /5ml and 20ml sterile disposable syringes.
2. Penicillin G.vials 10 lac units/vial x 5 vials 3)
Water for injection 500 ml x 5 bottles.
4) 30 ml vials 25 autoclaved. (empty xylocaine vials)
5) 5 ml vials with cap 50 autoclaved. (empty penicillin vials)

EXERCISE NO. 10

OBJECTIVES - STUDY OF ACTION OF ANTIDEPRESSANTS ON MICE

At the end of the practical session a student shall be able to:
1. Calculate the exact dose and volume to be administered to an animal when given relevant data.
2. Record and tabulate observations in a scientific manner and draw valid inferences.
3. List adverse effects of the commonly used anti-depressants.

Animals: Mice

Apparatus: Glass jar, syringes, feeding cannula

Drugs and solutions:

Imipramine	50mg / 3ml
Normal saline	0.9%

PROCEDURE

Weigh two mice and number them. Administer 0.5ml of imipramine to one mouse and 0.5ml of normal saline to the other mouse orally. After one and a half hours the mice are plunged individually into a vertical glass jar filled with water. Observe the behaviour of the mice for three minutes. Note the total duration of immobility during this period.

Pool data from other groups and tabulate. Apply a suitable statistical test and write your conclusions based on the results of the test. After the experiment is completed conduct a small group discussion on the commonly used antidepressants, the adverse effects and limitations of the drugs.

Forced Swimming Test Model:

This model is not a replica of depression that occurs in human beings. The animal is forced to swim in a jar containing water from which it cannot jump. After swimming for some time, it remains immobile. This is called as behavioural despair. The mice are allowed to swim for a period of 3 mins and the time during which it remains immobile is recorded. This behavioural despair is equated with depression in human beings. Almost all available antidepressant drugs give positive results with this test. When the animal is pretreated with an antidepressant the animal will continue to swim for most of the 3 min period. False positive and false negative results can be obtained. Sometimes drugs which have antidepressant action in this model may not have the same effect in humans and vice versa. At the end of the experiment conduct a discussion on adverse effects of antidepressants.

Other models of depression:
Reserpine induced depression
Repeated noxious shocks model (learned helplessness)
Mother infant separation

EXERCISE NO. 11

OBJECTIVES - STUDY OF ANORECTIC AND LOCOMOTOR ACTIVITY OF AMPHETAMINE AND FENFLURAMINE

At the end of the practical class the student shall be able to:

1. Observe and record the changes in locomotor activity & food intake induced by amphetamine and fenfluramine.
2. Appreciate the need to treat obesity by changes in life-style.
3. Understand the limitations of treating obesity with anorectic agents.

Animals: Mice

Apparatus: Plastic animal cages - 35cm x 23 cm

Drugs and solutions:

Amphetamine sulphate = 0.5mg/ml
Fenfluramine = 1.0mg/ml
Normal saline

PROCEDURE

LOCOMOTOR ACTIVITY: Take 6 mice which have been fasted overnight and weigh them. Mark the bottom of the cages into 5 x 3 squares (15 in no.). Place one mouse at a time in a cage. Allow it to acclimatize to the cage for 3 minutes. Then record the number of times the mouse crosses a square over a 5-minute period. Also record the number of rearing and grooming movements during this period. Repeat with all the mice. Inject amphetamine sulphate 5mg/kg ip, fenfluramine 10mg/kg ip and normal saline 10ml/kg ip into two mice each. After 15 minutes again record the crossing over, rearing and grooming activity of each mouse over a 5-minute period.

ANORECTIC ACTIVITY: After noting the locomotor activity, place the two mice which have received saline in one cage, the mice which received amphetamine in another cage and the mice which received fenfluramine in a third cage. Place 10 grams of broken food pellets in each of the cages. Note their feeding behaviour for one hour. Then collect the remaining food pellets from each of the three cages and weigh them separately to quantify the amount of food consumed by both the mice in each group. Record your observations in a table.

Tabulate your findings after pooling data from all groups. Apply a suitable statistical test for the experiment on locomotor activity. Draw conclusions and write inferences. A group discussion on the non-drug treatment of obesity and the limitations of anorectic agents should follow.