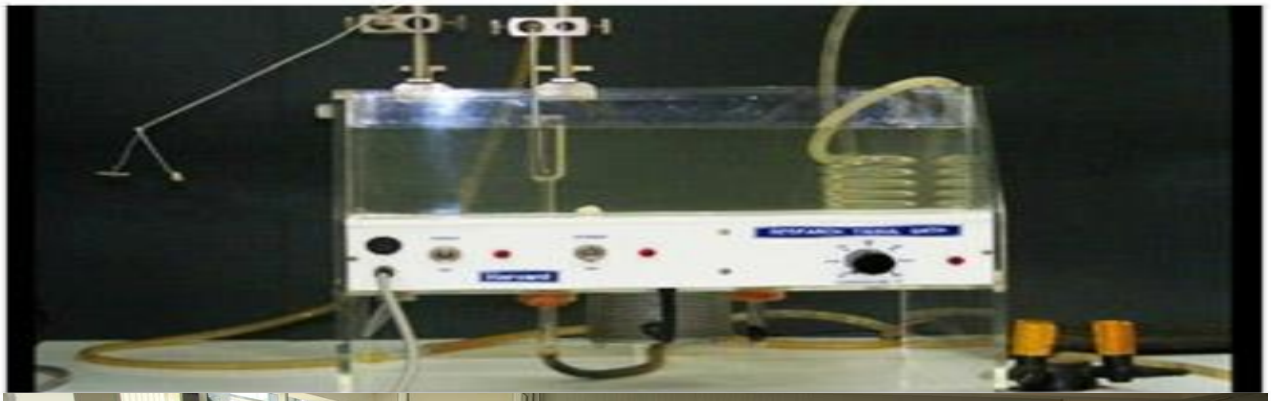
Lab Manual
Pharmacology lab
Women Dental College Abbottabad



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Description:

Pharmacology subject imparts knowledge and skills about the journey of the drugs starting from their origin to administration, their movement in the body (pharmacokinetics) and its beneficial and hazardous effects on our body (pharmacodynamics).

Pharmacology is the study of how a drug affects a biological system and how the body responds to the drug. The discipline encompasses the sources, chemical properties, biological effects and therapeutic uses of drugs. These effects can be therapeutic or toxic, depending on many factors. The pharmacology lab consists of equipment to investigate the activity of various drug samples.

The purpose of pharmacology is to determine the effectiveness and safety of drugs designed to treat, prevent, or diagnose disease. This includes the drug dosages, frequency of dosages, adverse effects, and how the drugs effect different populations of people.

Pharmacology subject imparts knowledge and skills about the journey of the drugs starting from its origin to administration, its movement in the body (pharmacokinetics) and its beneficial and hazardous effect on our body (pharmacodynamics).

Our lab is equipped with sophisticated instruments. Students will be able to learn about calculations of drug doses and the mechanism of action of drugs. It also includes pharmacological screening

The Department of Pharmacology plays an important role in the educational and research missions of WOMEN DENTAL COLLAGE ABBOTTAB. The Department is fulfilled by its experienced teaching faculty with a dedicated vision of research and outcome-based teaching. The department is well known for its educational activities, infrastructure and well-maintained animal house.

Animal House

Animal House facility at WOMEN DENTAL COLLAGE ABBOTTAB for the Purpose of Control and Supervision of Experiments on Animals. All activities to laboratory research are performed as per the guidelines of institute. The facility is equipped with animal rooms for separate species, quarantine, and experimental area.

Undergraduate students are familiar in animal handling and evaluation of drugs by in-vivo and in-vitro models. Our students are actively involved in research activities and acquiring knowledge through participating in various skill development programme and workshops

Aims and Objectives:

The main objectives of pharmacology lab

1. Develop good understanding of undergraduate students and health professionals learn the correct knowledge of lab practice of pharmacokinetics and pharmacodynamics of drugs.
2. It also helps to measure students' progress in lab learning as they gain confidence in the lab experimental skill

3. Ensure the good handling practice of experiment.

The mission of the laboratory is to promote experimental lab competence and enhance the skills of medical students (undergraduate 2ndyear BDS) during their training.

Faculty Responsible for Course Conduction:

2nd Year BDS
Pharmacology
(Dental Material Medica)

Overview

Program	Bachelor of Dentistry
Course Name	Dental Material Medica
Contact Hours	200 hrs
Infrastructure Requirements	Lecture hall, labs animal house musiam

Faculty Responsible for Course Conduction:

Sr. No	Faculty	Designation
1	Dr Gul Mehnaz	Associate Professor/HOD
2	Dr Sahar amin	Demonstrator
3	Dr Dur-e-saman	Demonstrator

Details of Supporting Staff:

Sr. No	Staff	Designation
1	Dilawar Shah	Lab Attendant
2	Miss Nosheen	Computer operator
3	Tamuor	Office Boy
4	Yasir Hussain	Office Boy

Kymograph and Organ Bath Assembly

The most commonly used equipment in undergraduate pharmacology laboratory to study the effects of various drugs on GIT, isolated tissues and heart is kymograph attached with organ bath assembly.

1. Kymograph

Kymograph is an apparatus consisting of a rotating drum for recording wavelike motions, variations, or modulations, such as muscular contractions. Kymograph is generally used to study the effects of drugs on tissue preparations. It is a standalone recording apparatus used alongside other apparatus such as organ bath. Writing levers are used to trace the recording from muscle contractions. It mainly consists of following parts

Cylinder Clamp: It is a lock, which is used to fix the drum on the central shaft so that the drum and the cylinder shaft rotate together.

Drum: A metallic cylinder covered with a paper for recording the activity.

Spindle: A metallic rod at the top of the kymograph that holds the drum and the shaft.

Kymograph Arm: A moveable, adjustable arm on the top of kymograph.

Main Switch: Present on the front of the kymograph, directly connected to the socket of external switch.

Drum Motor Control Switch: Starts/stops the drum motion with an indicator light.

Speed Regulator: Are used to adjust the speed of the drum. The drum should be moved manually only in neutral position, indicated by N on the gear. **Start/Pause lever:** A small lever connected with the drum movement. If it is at the "start" position drum moves freely, and if it is at "pause" position, the drum takes one rotation and then stops.

KHMOGRAPH

Organ Bath

EXPERIMENTS

The organ bath assembly is a traditional experimental set-up that is commonly used to investigate the physiology and pharmacology of in vitro tissue preparations. Perfused tissues can be maintained for several hours in a temperature controlled organ bath. It has following main parts.

Organ bath: It is a hollow glass tube that is connected with inlet and outlet tubes and is fixed in the center of the outer water bath. It may have varying capacity, generally from 10 to 50ml. Physiological solution is added into bath through the inlet tube (coiled around the tissue bath forming outer jacket) from a reservoir. The coiling tube makes the solution warm and maintains the temperature inside the organ bath. The organ bath is drained out by opening the outlet stop cork when washing of the tissue is required and fresh solution is filled into the organ bath by opening the stop cork of inlet tube from the reservoir.

Organ Bath Assembly

Procedure

1. Adjust the height of the drum on spindle by losing the clamp and tight the cylinder clamp.
2. Paste graph paper on the drum smoothly.
3. Connect the instrument to the power supply.
4. Adjust the speed by positioning the gear and the speed-knob appropriately.
5. Switch on the main supply to start the drum rotation.
6. Adjust the bath assembly to the adjustable arm.
7. Mount the given tissue preparation in the bath assembly.
8. Attach the pen assembly with writing lever arm and adjust the position of adjustable arm with respect to the drum properly.
9. Fill up ink in the stylus
10. Record the tissue activity according to, the requirements of the experiment.

Precaution

Don't pause for a long time because it stops the drum immediately while the motor inside remains on and could be burnt.

Metrology

Metrology is defined by the International Bureau of Weights and Measures (BIPM) as "the science of measurement, embracing both experimental and theoretical determinations at any level of uncertainty in any field of science and technology".

Importance of Measurement Systems

One of the most essential functions of a Medical professional is the ability to perform accurate pharmaceutical measurements, calculations and conversions. Without this ability, a medical professional is not able to apply their knowledge of pharmacology in a practical manner during their everyday work functions. This is important as one incorrect calculation, conversion or measurements will affect a dosage, and can potentially harm a patient. Possessing a working knowledge of the pharmaceutical systems of measurement will benefit a medical professional. There are three measurement systems in pharmacy calculations, which a medical professional must learn to carry out the critical functions used in the pharmacy

Metric System

The metric system is a decimal system with all multiples and divisions based on a factor of 10. This system is also the most commonly used system of measurement for pharmacy calculations, as it allows for quick and easy conversions between different systems of measurement. As the metric system is based on multiple of 10 so following prefix are used:

Deca = 10 times
Hecta = 100 times
Kilo = 1000 times
Mega = 1000000 times
Deci = 1/10
Centi = 1/100
Milli = 1/1000

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Micro = 10^{-6}
Nano = 10^{-9}
Pico = 10^{-12}

Apothecary System

The apothecary system is one of the three systems of measurement used in pharmacy calculations, which uses weight and volume as divisions of measurement. This includes measurements of ounces, gallons, pints and quarts.

1 fluid ounce = 29.573 milliliters (mL)
8 fluid ounces = 1 cup = 16 tablespoons (tbsp)
2 cups = 1 pint = 473 ml
2 pints = 1 quart = 0.946 liter
4 quarts (qt) = 1 gallon = 3.785 liters (L)
1 tablespoon = $1/2$ fluid ounce = 3 teaspoons

Solutions

Study of different biological Salt Solutions used in vitro studies and their functions and composition

Biological solution is an artificially prepared solution similar to blood plasma in salt composition and osmotic pressure. Biological solutions are used in physiological experiments with isolated

organs and in clinical practice. It is important to select a particular solution in which a tissue survives longest. The functions of a salt solution are:

1. To maintain the medium within physiological pH range
2. To maintain intracellular and extra cellular osmotic balance
3. Modified with a carbohydrate, such as glucose serves as an energy source

Following biological solutions are used commonly in laboratory.

1. Tyrode's solution

Tyrode solution is a solution that is roughly isotonic with interstitial fluid and is used in physiological experiments and tissue culture. It resembles lactated Ringer's solution, but contains magnesium, a sugar (usually glucose) as an energy source and uses bicarbonate and phosphate as a buffer instead of lactate. It is used to perfuse intestine.

2. Ringer's solution

Ringer solution is a solution of several salts dissolved in water for the purpose of creating an isotonic solution relative to the body fluids of an animal. Ringer's solution typically contains sodium chloride, potassium chloride, calcium chloride and sodium bicarbonate, with the last used to balance the pH. It is used for amphibian tissues; i.e. frogs.

3. Kreb's Solution

It has been used experimentally, for instance to study arteries *ex vivo* and during isolated muscle testing of mammalian skeletal muscles.

Handling of Laboratory Animals

RABBIT EXPERIMENT

Material Required

Rabbits, Rats, Gloves

The most commonly used animal in undergraduate pharmacology laboratory are rabbits and rats. It is very important to learn the animal handling properly to carry out the practical and reproduce the results.

Rabbit Handling

Animals should be handled 0nl y in the animal house. Always wear lab coat before handling the animals. Do not panic the animal and grasp the animal gently without harming.

1. Rabbits can be grasped by gripping the scruff of the neck firmly with one hand and their body with the other hand or they may be held in a wooden animal box for the purpose of injections. If the animal is frightened, put a cloth around the legs to prevent scratching (Fig. 12a).
2. The area should be rough, in case of slippery area, the rabbit may get irritated due to
3. slipping of feet. Hold the animal firmly and gently and never let it go if the animal get

frightened or irritated.

4. If the rabbit is in a box which restrains movements without undue pressure, then only one person is needed for giving injection or instillation of eye drops. Sometimes if the animals are much frightened and aggressive, it is advisable to wear the woolen gloves.

Effects of Drugs on Rabbit's Eye

Principle:

The parameters like corneal reflex, light reflex, and pupillary diameter are studied before and after adding drug.

1. Corneal reflex is studied by touching the cornea with a cotton probe. The rabbit immediately
2. closes the eye lid.
3. The light reflex is studied by directing the light rays into the rabbit eye and pupillary
4. diameter is reduced.
5. The diameter of the pupillary size is measured by a scale

Apparatus:

Scissors, Scale, Dropper, Torch, and Cotton plug.

Subject:

Rabbit

Drugs:
Pilocarpine, Atropine, Phenylephrine, Proparacaine

Procedure:
1. Hold the animal from trunk
2. Trim the eye lashes of both eyes with scissors
3. Consider one eye as control and other as experimental
4. Test and record the size of pupil, color of conjunctiva, corneal reflex and light reflex of both eyes of the rabbit
5. Then tilt the head of the animal to opposite side and block naso-lacrimal duct with thumb
6. Put a few drops of drug in experimental eye
7. Allow the drug to be absorbed fully in eyes
8. Then record the observations after 0, 5, 10, 15, 30 and 45 min and tabulate the results.

Precautions:
1. Care should be taken not to injure the eyes
2. Before cutting, moisten the eye lashes to avoid eye lashes from getting into eye of animal
3. Mark one eye as standard/control for comparison with experimental
4. Block the naso-lacrimal duct for 1-2 min to avoid drainage of drug into nose
5. Head of the animal should be tilted backwards allowing the drug to remain in the eye for few
6. min for its adequate absorption and action
7. The observation should be taken in dim light and entry of light rays directly into the eyes should be avoided.

Observations & Calculations

1. Pilocarpine HCL:

Time (min)	Size of Pupil (mm)		Light Reflex		Corneal Reflex		Conjunctival Color		Dryness of Eye	
	C	E	C	E	C	E	C	E	C	E
0										
5										
10										
15										
30										
45										

To Study of the Effects of Cholinergic Drugs with Anticholinergic Drugs (antagonism) on Rabbit's Intestine

Apparatus:
Dissection box, kymograph, smoke drum, isolated organ bath, petri-dish, thermometer, syringe, beakers, stitching needle, thread, stop watch, water bath, funnel.

Subject: Rabbit

Physiological salt solution: Tyrode solution

Drugs

Acetylcholine, Atropine **Procedure**:

1. Set kymograph and organ bath on table, following the washing of organ bath. Organ bath consists of two jackets; outer and inner. Fill outer jacket with water and turn it ON. Maintain the temperature at 38-40°C. Then fill the inner jacket with Tyrode solution and mark the level. Maintain this level and temperature throughout the experiment. Aerate the Tyrode solution with oxygen continuously.
2. Dissect the rabbit and dissect out the rabbit intestine, cut a piece of rabbits intestine 2cm in length and keep it in petri dish containing Tyrode maintained at temperature of 37 ^{0}C. Clean and remove the fecal matter from this tissue. Then pass a thread through one end of tissue and give a knot. Similarly pass another thread from other end of this tissue and tie it.
3. Now suspend this tissue in inner jacket of organ bath with the help of tissue suspending hook by tying one end of thread (loop) with hook and other with the lever then adjust the liver so that it moves smoothly against the kymograph drum. Wash the tissue by replacing the Tyrode solution and allow it to take rest for at least 2-3 min. before the start of experiment. Take muscle contractions without adding any drug until it becomes regular and uniform.
4. Add few drops of acetylcholine into inner jacket of the organ bath and record the muscle contraction for few seconds.

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5. Rinse the tissue with temperature maintained (37 ^{0}C) Tyrode solution until contractions reaches to baseline.
6. Add few drops of acetylcholine, at the height of contraction, add the atropine and record the contractions.

Precautions:

1. Wash the inner jacket at least 2 to 3 times with Tyrode solution
2. Temperature of outer and inner jacket should be consistently maintained throughout the
3. experiment at 37 ^{0}C
4. Clean the tissue properly to avoid the damage of enteric plexus
5. Tissue should be suspended vertically in such way to avoid direct contact with inner
6. walls & suspended loop
7. Keep the tissue all the time in Tyrode solution
8. Maintain the level of Tyrode soln. before & after washing
9. Dosing interval for rabbit intestine is 3 mins
10. Different syringes should be used for different drugs

¬ No air bubble should present during measuring the dose of drug

Format: Traditionally a prescription is written in a definite order that facilitates its interpretation. Prescription begins with name, age, address, and diagnosis of the patient on the left hand side and date on the right side.

Parts of prescription

Main body of prescription consists of following parts

1. Superscription
2. Inscription
3. Subscription
4. Signatura
5. Physician's signature

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1. Superscription

This is the sign Rx which is instruction to the pharmacist. It is derived from Latin word recipe, meaning "take thou".

2. Inscription

It is the main part of the prescription that contains name of drug, dosage form and strength.

3. Subscription

It contains direction for the pharmacist including size of each dose, amount to be dispensed and form of drug.

4. Signatura

It contains direction for the patient, usually precede by the symbol Sig: the place where the physician indicates instructions. These may include (a) The method of administration and application (b) The dose if the preparation is for internal dose (c) The time of administration or application

(d) The diluents (e.g. water) if relevant, or means of application (e.g. brush) (e) The part of body where the preparation is to be applied, in case of external use

5. Physician's signatures

These are written on the right hand side at the end of prescription. It includes; i). Physician signature.

ii). Professional qualification.

iii). License Number iv). Address

General considerations

1) It should be in National language.
2) It should be legible.
3) Abbreviations should be avoided.
4) Preferably the doses should be written in metric system.
5) Medicines should be written in serial number.
6) Every prescription should be signed.
7) Form & stregtn of every medicine should be written.
8) Full name rather than formula of the medicine should be written.
9) Instruction to patient for intake of medicine should be complete.
10) Instruction regarding diet & medicine should be complete.
11) Instruction regarding diet & medicine should be written at the end.

Prescription Writing

Prescriber's Name:
License No:
Timing:
Contact No:

Patient's Information

Name _____ **Age:** _____

Gender_____ **Weight**_____

Address _____ **Date:** _____

Presenting Complains

History

℞

Diagnosis

Refill Information

Sig.----------

WOMEN MEDICAL COLLEGE ABBOTTABAD
DEPARTMENT OF PHARMACOLOGY

LIST OF CHEMICALS.

S.No	NAMES OF CHEMICALS
1	Ammonium Chloride
2	Aspirin powder
3	Caffeine powder
4	Calcium chloride

5	Ext.Glycyrhiza
6	Glucose Mino Hydrate
7	Iiodine crystal
8	Eye drops
9	Magnesium chloride
10	Potassium Permanganate
11	Potassium Citrate
12	Potassium Iodide
13	Paracetamol Tablets
14	Potassium Chloride powder
15	Sulphur powder
16	Spt.Ammonia Aromatic
17	Sodium Citrate
18	Syrup Aurantia
19	Sodium Chloride powder
20	Tincture Card co
21	Vaseline
22	Zinc Oxide
23	Sodium Bicarbonate
24	Pilocarpine Eye Drop
25	Atropine Eye Drop
26	Acetylcholine powder
27	Barium Chloride
28	Distilled Water
29	Ephedrine Powder
30	Lignocaine Eye Powder
31	Histamine Powder
32	Antihistamine

WOMEN MEDICAL COLLEGE ABBOTTABAD
DEPARTMENT OF PHARMACOLOGY

LIST OF INSTRUMENTS.

S.No	INSTRUMENTS	QTY
1	Electronic Balance	2
2	Physicial Balance with weight box	6
3	Double Tissue Organ Bath	6
4	Kymograph	14
5	Water Bath	1

6	Weighing Machine.	1
7	China dish	16
8	Mortar with pestle	14
9	B.P Apparatus	5
10	Stethoscope	6
11	Torch with cell	9
12	Scissors	6
13	Stop watch	4
14	Beakers (1000ml)	6
15	Beakers (500ml)	2
16	Beakers (250ml)	2
17	Beakers (100ml)	1
18	Graduated Cylinder (100ml)	7
19	Graduated Cylinder (50ml)	6
20	Graduated Cylinder (10ml)	7
21	Petri Dish	12
22	Dispensing bottles	8
236	Eye drop bottles	8
24	Ointment box	10
25	Ointment Slabs	6
26	Spatula	6
27	Pipettes (10ml)	2
28	Dissection box	2
29	Pinch cock	6
30	Screw pinch cock	6
31	Artery Forceps	12
32	Plane Forceps	12
33	Frog board	1
34	Paper folder	7
35	Glass bottles(250ml)	22
336	Spirit Lamp	8
37	Oxygen cylinder	3
38	Wrench	1
39	Syringes (5ml,1ml)	50 Nos
40	Distillation Apparatus	1
41	Thermometers	14
42	Operation table	1

OBJECTIVES AND LEARNING STRATEGIES/TOS

S.NO	Topic Practical work	Teaching Hours (100hrs)	Mode of Teaching	Assessment Tools
	Terminology and branches of pharmacology Metrology Medical Abbreviations Pharmaceutical procedure P-drugs Problems 1-8 Writing prescriptions Prescription of pharyngitis Prescription of infective diarrhea Prescription of Baciliary dysentery Prescription of ameobic dysentery Prescription of enteric fever Prescription of Round Warm Prescription of hook warm Prescription of Tape warm Prescription of UTI Prescription of Iron deficiency anemia Prescription of Acute Malaria Prescription of Chloroquine resistant malaria Prescription of Acute Bronchitis		Practical	OSPE

	Prescription of Congestive Heart Failure Prescription of Angina pectoris Prescription of Pulmonary Tuberculosis **Solutions** To prepare and dispense 70ml of Normal Saline -0.9 percent solution To prepare and dispense 60ml of 5 percent dextrose saline solution. To prepare and dispense 90ml of 0.0 percent KMNO4 solution To prepare and dispense 20ml of lugol,s iodine solution. To prepare and dispense 70ml of Normal Saline -0.9 percent solution **Mixtures** To prepare and dispense 30ml of Sodium Salicylate Mixture. To prepare and dispense 40ml of Mixture Saline Purgative **Powders** To prepare and dispense powders of Aspirine, Paracetamol, Caffine). To prepare and dispense powders of Aspirine, Paracetamol, Caffine). To prepare and dispense 2 powders of ORS each to make of 100ml of solution, **Reflexes of Eyes** To Demonstrate the effect of anti-cholinergic of drug 1% Atropine in Atropine in Rabbit,s Eye.			

	To Demonstrate the effect of cholinergic of drug 1 % Pilocarpine on Rabbit,s Eye. To study the effect of various of drug on Rabbit,s Eye. To Demonstrate the effect 2 % ephidrine solution on Rabbit,s Eye. To Demonstrate the effect of local anesthesia (1 % lignocaine) on Rabbit,s Eye. Effect of Atropine on Human subject. Demonstrate effect of Adraline on Human subject. **ORGAN BATH** **To** observe the phenonmenon of Antagonism between Histamine and Anti-histamine on piece of Rabbits intestine. To Demonstrate Antagonism B/W cholinergic & Anti-cholinergic drug on isolated piece of small intestine. Introduction to Route of Drugs administration . To study intravenous route of Adminstration. To demonstrate the IM Route Adminstration. To demonstrate sub-cutaneous Route of drug Adminstration. To demonstrate the use of inhaler and Spacer Devices.			

	To study the parts & Working of IV infusion set-up.			

Prepared by

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