East Point College of Pharmacy

East Point Campus, Jnana Prabha, Virgo Nagar PostBengaluru – 560049, Karnataka

Approved
by
Pharmacy Council of India, New Delhi

Affiliated
to
Rajiv Gandhi University of Health Sciences
Karnataka Bengaluru – 560 041
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LAB MANUAL

PHARMACOLOGY - II

B. PHARM 5TH SEMESTER

B Pharmacy

Program Outcomes (PO's)

PO 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.

PO 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.

PO 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

PO 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.

PO 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 wellbeing.

PO 6- Professional Identity

Understand, analyse and communicate the value of their professional roles in society (e.g. health care professionals, promoters of health, educators, managers, employers, employees).

PO 7- Pharmaceutical Ethics

Honor personal values and apply ethical principles in professional and social contexts. Demonstrate behaviour 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

PO 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

PO 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.

PO 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.

PO 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-access and use feedback effectively from others to identify learning needs and to satisfy these needs on an ongoing basis.

Programme Specific Outcomes (PSO's)	
PSO 1	Acquire a thorough foundational knowledge in pharmaceutical sciences, including pharmacology, pharmaceutics, medicinal chemistry, and pharmacognosy, to excel in further academic pursuits
PSO 2	Gain expertise in the application of contemporary pharmaceutical techniques and technologies, enhancing employability across various sectors including the pharmaceutical industry, academia, and research institutions.
PSO 3.	Equip with entrepreneurial skills and knowledge of pharmaceutical business management, including market analysis, product development, regulatory affairs, and financial planning, to initiate and run successful ventures in the pharmacy sector

Course Outcomes (CO's)
Code: BP507P Pharmacology- II
CO 1: Understand the mechanism of action and its relevant in the treatment of different disease.
CO 2: Demonstrate isolation of different organs/ tissues from the laboratory animals simulated experiments.
CO 3: Demonstrate the various receptor action using isolated tissue preparations.
CO 4: Appreciate correlation of pharmacology with related medical science.

Experiment No: 01

INTRODUCTION TO *IN-VITRO* PHARMACOLOGY ANDPHYSIOLOGICAL SALT SOLUTIONS.

Aim: To study the Introduction about In-Vitro Pharmacology and Physiologicalsalt solutions.

Introduction:

"In vitro" is a Latin word that means "**within the glass**" Therefore the studies which are done outside the living organism, inside glass (test tubes or Petri dishes) are known as In vitro studies.

It is the experiment or observations done on the tissue outside of the living organism in a controlled environment, usually using Petri dishes and test tubes. In vitro processes, conditions are artificial and they are reconstructions of In vivoenvironments. Artificial conditions are formed by mixing the necessarycomponents and reagents under controlled conditions inside a glassware in thelaboratory.

In vitro methods are widely used in pharmaceutical industry to produce large scale pharmaceuticals using microorganism due to ease of production and economic benefits.

Advantages of in-vitro studies:

In vitro studies permit a species-specific. simpler, more convenient, more detailedanalysis

In vitro studies replace studies in whole animals. It is less expensive andprovides quicker results.

Definition:

Estimation of potency of an active ingredient in unit quantity of preparation is detection and measurement of concentration of substance preparation usingbiological method is known as bioassay.

Principle of Bioassay:

The basic principle of bioassay is to compare the test substance with internal standards of the same and find out how much test substance is required to producesame biological effect that are produced by the standard.

Methods of bioassay:

1. Quantal assay
2. Graded response assay

1. Quantal assay:

In this bioassay the dose of the standard thar of the unknown which provide the predominant or all or none, response is measured and their potency ratio is compared.

Eg: digitalis induced cardiac arrest is in guinea pig or cat.

2. Graded response assay:

These are called graded response assay because the response to varying doses ofdrugs is graded and measured repeatedly of acetylcholine on frog rectus abdominal muscle.

2.1. Matching bioassay:

It is used when the test sample is too low. In this method a curve of which matchis response with dose of standard is found by trial and error. From this the potencyof unknown test solution can be calculated.

2.2. Bracketing Bioassay:

It is also used when the test sample is too small the observed test response with the drug is tracked between the one higher and one lower response of standard. The strength of the unknown can be found by sample interpolation of this trackedresponse on the dose

2.3. Interpolation method:

It is based on the principle of log response. In the method LDR curve of the standard drug is obtained at 1st later 2nd and 3rd response of the unknown which fall in between the linear portion of the LDR curve are obtained by trial and error.

Then interpolation of this response of log dose axis taking the antilog the concentration of this response can be found.

2.4. Multiple point bioassay:

a. Three point bioassay:

The response of standard drug and one response is due to the test sample are takeninto consideration. The test response should be intermediate between the 2 responses of the standard.

b. Four point bioassay:

Two response of standard drug and two response of test drug are made use shouldbe linear portion of the concentration curve and also the ration between the dose should be perfectly 1:2 the solution of test response are recorded in the random fashion.

Instruments used in In-Vitro Pharmacology

1. Dale's organ bath or isolated organ bath.

a. Inner glass tube or organ bath containing PSS and tissue

b. Connected reservoir through polyethylene or rubber tube.

c. An outer glass or filled with water

d. Kymograph drum

2. Sherington's research kymograph:

a. Base hoof (Legs)

b. Slide hoof

c. Gear rods

d. Drum cylinder.

3. Lever

It is the device by virtue of which response of isolated tissue can berecorded and magnified.

a. Fulcrum

b. Stylus

Magnification: The fulcrum should be placed that there is some magnification ofthe actual concentration. In order to achieve this distance between the writing point and the fulcrum and point of attachment of tissue.

Name of tissue magnification:

(i) Guinea pig ileum 5-10 times.

(ii) Rat uterus 4-6 times.

(iii) Frog rectus, abdominus muscle 10 times.

(iv) Rat fundus stripe 15 times.

Different types of levers:

1. Simple lever
2. Frontal writing lever
3. Starling's heart lever
4. Universal lever
5. Arterial cannula
6. Venus cannula
7. Tracheal cannula
8. Bull dog clamp
9. Rat holder
10. Syringe

Physiological salt solutions

The ionic requirements and nutritional supply can be provided by using the suitable solution, commonly known as physiological salt solution. Also called asPSS /Ringer solution Its composition is such that it provides an artificial media resembling the inorganic composition of blood plasma together with a buffer mechanism to maintain the optimum pH about 7.0 to

7.2 and glucose to facilitate tissue metabolism.

Commonly used PSS

1. **Frog ringer- (**For heart, rectus abdominis and other preparations of frog)
2. **Tyrode** (For guinea pig ileum, rat ileum, rabbit ileum etc.)
3. **De Jalon** (For rat uterus)
4. **Kreb's Solution** (for rat funded strip, Tracheal preparations, Vas deferens)etc.

The PSS should:

Maintains tissue outside the animal body. Select PSS in which tissue lastslonger.

Prepare the solution with the help of distilled or double distilled or deionized

water. Prepare fresh solution.

Ingredients	Concentration in gms/l			
	Frog ringer	Tyrode	Kreb's	De jalon
NaCl	6.5	8.0	5.9	9.0
KCl	0.14	0.2	0.35	0.350
$CaCl_2$	0.12	0.18	0.28	0.003
NaH_2PO_4	0.05	0.1	-	-
KH_2PO_4	-	-	0.16	-
$MgSO_4$	-	-	0.11	-
$MgCl_2$	-	0.1	-	-
Glucose	1.5	1.0	2.0	0.5
$NaHCO_3$	0.4	1.0	2.1	0.5

Purpose of each ingredient

1.	Sodium Chloride (NaCl)	To maintain iso osmolarity, isotonicity,excitability, contractility of the preparation
2.	Potassium Chloride (KCl)	To maintain ionic balance
3.	Calcium Chloride ($CaCl_2$)	To maintain the contractility of the preparation
4.	Sodium bicarbonate ($NAHCO_3$)	To provide alkaline pH
5.	Glucose	To provide energy
6.	Sodium or potassium	Act as the buffer
7.	Di hydrogen phosphate	To stabilize the preparation
8	Sulphate	To stabilize the preparation and hence to reduce the spontaneous activity

Experiment No:02
EFFECT OF DRUGS ON ISOLATED FROG HEART

Aim: To study the effect of drugs (inotropic and chronotropic actions) on perfusedfrog heart.

Principle: Drugs may influence the rate (chronotropy) and force (inotropy) of contraction of the heart. An increase in the heart rate is called a "positive chronotropic" response, while a "negative chronotropic" response is a decrease in the heart rate. Similarly, an increase in the force of contraction is called a 'positive inotropic' response, and a decrease in the force of contraction is called a 'negative inotropic' response.

Sympathomimetic amines such as adrenaline and noradrenaline produce positive inotropic and positive chronotropic response. Whereas parasympathomimetic such as acetylcholine produce negative inotropic and negative chronotropic response.

Requirements:

Animal	: Frog
Apparatus	frog's ringer solution, reservoir, tubing, screw clip, cannula, clamp, boss- head, thread, syringe and needle.
Drugs	: Adrenalin (stock solution 10 µg/ml)

Noradrenaline (stock solution 10µg/ml) Acetylcholine (stock solution 10µg/ml) Calcium chloride (stock solution 10µg/ml)

Potassium chloride (stock solution 10µg/ml) Physiological solution: Frog ringe

Procedure:

1. Pith the frog and pin it to the frog board.

2. Give a mid-line incision on the abdomen. Remove the pectoral girdle andexpose the heart.

3. Carefully remove the pericardium and put a few drops of frog ringer over the heart.

4. Trace the inferior vena cava, put a thread around it, and give a small cut in order to insert the venous cannula which is in turn connected to a perfusion bottlecontaining frog ringer. Insert a cannula in the vein and tie the thread to assure thecannula in place.

5. Give a small cut in one of the aortae for the perforate to come out.

6. Adjust a proper venous pressure of 2-4 cm by altering the height of the perfusion

7. bottle. The effective venous pressure is the height in cm from level of the venous cannula and the ringer level in the perfusion bottle. The use of Marriott's bottle helps in attaining the constant pressure. Start the perfusion by opening the screw clamp attached to the tube.

8. Pass a thin pin hook through the tip of the ventricle, and with the help of afine thread attached to the hook, tie it to the free limb of the universal lever, whichis fixed to a stand. Adjust proper tension and magnification by altering the heightof the lever. Record the normal contraction of the heart on the smoked drum.

9. Inject 0.1,0.2,0.5 and 1 ml of the stock solution of each drug in a sequentialorder and note the change in the rate and amplitude of contraction. Keep at least 5min gap between the administration of each dose of the drug. The drug is administered by injecting the drug into the perfusion tube very close to the venouscannula. Take precautions to avoid any leakage of the drug from the tube, and theinjection of air bubbles.

10. Label and fix the tracing with the fixing solution.

Observation:

Report:

Experiment No: 03

EFFECT OF DRUGS ON NORMAL AND HYPODYNAMIC PERFUSEDFROG HEART

Aim: To study the effect of drugs on normal and hypo dynamic frog heart.

Principle: The myocardial contraction of normal and hypodynamic frog heart takes place according to starling's law of heart. According to this law force of systolic contraction is directly proportional to the fiber length in diastole. Since systolic contraction represents cardiac output and the fibre length in diastole indicates venous pressure, the law indicates that cardiac output (i.e., Stroke volume) is directly related to venous return or venous pressure during diastole.

When the cardiac musculature fails to obey this relationship as in failing heart (i.e., Congestive heart failure) there will be decrease in stroke volume (cardiac output), incomplete emptying of the ventricles during systole and enlargement of heart size due to residual blood in the heart at the end of the systolic contraction. Whenthe heart is in this state i.e., inability to contract to physiological normal it is saidto be a hypodynamic heart. Experimentally hypodynamic heart can be produced by perfusing the heart with ringer containing less quantity of Calcium as this bivalent ion is essential for myocardial contraction.

Requirements:

Animal : Frog

Drugs : Digitalis (Digoxin stock solution 50µg/ml) Calcium chloride (stocksolution 100 µg/ml)

Physiological solution Normal frog ringer and frog ringer containing ¼ $CaCl_2$.

Procedure:

1. Set up the perfusion of frog heart with normal frog ringer solution as described inthe earlier experiment.

2. Record the effects of (0.1,0.2,0.4, and 0.5 ml) digoxin and $CaCl_2$ (0.1,0.2,0.4, and 0.5 ml). Note the dose that gives an adequate response.

3. Replace the perfusion fluid with modified Ringer containing only ¼th the Calcium chloride as compared to that of normal ringer. Note the change in the pattern of the recording of the heart.

4. When the heart is depressed markedly in presence of modified Ringer, administer digoxin (0.1,0.2,0.4 and 0.5 ml) and CaCl2 (0.1,0.2,0.4 and 0.5 ml). Note the change in contractility.

5. 5.Fix the tracing and compare the responses of these drugs in normal andhypodynamic heart.

Observation:

Report:

Experiment No: 04

EFFECT OF DRUGS ON BLOOD PRESSURE AND HEART RATE OFDOG

Aim: To study the effect of drugs on **blood pressure** and heart rate of dog.

Principle: The arterial blood pressure is defined as the pressure exerted by the blood on the walls of the blood vessels. Therefore,

blood pressure = cardiac output × peripheral resistance. The heart and the blood vessels are under the control of autonomic nervous system.

Both sympathetic and parasympathetic nerves supply the heart. Parasympathetic innervations are through the vagus supply the heart. Parasympathetic innervations are through the vagus whereas the sympathetic nerve supply to the heart comes from fibres arising from stellate or inferior cervical ganglion. The nervous supplyto the blood vessels is principally from the sympathetic system. In general, sympathetic stimulation (administration of adrenaline and noradrenaline) increases cardiac output and resistance to flow leading to an increased blood pressure.

On the other hand, parasympathetic stimulation (administration of acetylcholine) decreases cardiac output which lowers the blood pressure.

Requirements:

Animal – Dog (6-8 kg)

Anesthetic pentobarbitone sodium (45 mg /kg, iv; prepare a stock solutioncontaining 45mg/ml of the drug and per kg of body weight.

Drugs: adrenaline, noradrenaline, isoprenaline, acetylcholine (all 100 mg/mlstock solution), normal saline, and sodium citrate (10% w/v).

Equipment: Artery cannula, venous cannula, dog operating table, researchkymograph (big), mercury manometer, and surgical equipment.

Procedure:

1. Anaesthetize the dog with pentobarbitone (45 mg/kg) given intravenously.

2. Cannulate the femoral vein and connect it to a burette containing saline.Femoral vein cannulation is used for drug administration.

3. Cannulate carotid artery and mount the blood pressure using a mercury manometer.

4. Record the base –line mean blood pressure response. Note the heart rate.

Inject 3 or 5 µg/kg adrenaline through the femoral venous cannula. Note the sequence of the response, *i.e.*, rise in blood pressure, heart rate, the vagal notch, blood pressure falling below the base line and recovery to Pre-drug base line. Waitfor 5-10 min.

5. Similarly administer noradrenaline, isoprenaline and acetylcholine, one after theother. Give a sufficient gap (5-10 min) between the effects of two drugs.

Inference:

Report:

Experiment No:05

STUDY OF DIURETIC ACTIVITY OF DRUGS USING RATS/MICE. (LIPSCHITZ TEST)

Aim: To study the effects of various drugs (diuretics) on the output of urine.

Principle: Diuretics are the compound which increases the flow of urine. Normalurine output in rats is very small (1-2 ml/rat/day). Hence to get the measurable quantity the animals are first hydrated. The urine output is increased after administration of diuretics like urea, hydro flume thiazide and furosemide. An increase in volume of urine is measured with the help of a measuring cylinder andcompared with the normal urine output.

Requirement:

Rats, metabolic cages, graduated measuring cylinder. Drugs and solutions:Normal saline (0.9%), Urea (900 mg/kg; oral) Hydroflumethiazide (1mg/kg; oral) Furosemide (5mg/kg; oral)

Procedure:

1. Albino rats (150-200 g) are fasted (deprived of food and water) overnight and saline(25ml/kg) is administered orally with the help of oral feeding cannula.

2. Those animals are divided into four groups containing three rats in each as follows:

(i) Ist group- only normal saline (Saline)

(ii) IInd group- saline + Urea (900 mg/kg; oral)

(iii) IIIrd group-saline +Hydroflumethiazide (1mg/kg; oral)

(iv) IVth group-saline +furosemide (5mg/kg; oral)

3. After administration of drugs animals are placed in the four different metabolic cages.

4. Urine is collected in a measuring cylinder.

5. Time, when the first drop of urine is collected in a cylinder for each group is notedand the volume is recorded at intervals of 15 min for 3-4 hrs.

6. The difference in the volume collected at different time interval and total volumecan be compared with various diuretics.

Observation:

Report:

Experiment No:06

DRC OF ACETYLCHOLINE USING FROG RECTUS ABDOMINISMUSCLE

Aim: To record a concentration response curve of acetylcholine using rectusabdominis muscle preparation of frog.

Principle:

Dose (concentration) - response curves demonstrate graded responses to drugs oragonists where an increase in response is recorded with a subsequent increase inthe dose or the concentration of the drug. The dose –response curve is sigmoid orS- shaped. The first part (25% of graph) of the curve has poor discrimination between the doses. Whereas the middle portion of the curve shows greater sensitivity to different concentrations, and the responses to increasing concentrations are linearly differentiated. The last of the curve (plateau) shows the ceiling effect where no more increase in the response is seen with further increase in the dose.

When the doses are increased in geometric progression (logarithmic intervals) and the response is plotted against logarithms of doses, the relationship is called log dose-response curve.

Rectus abdominis muscle is a skeletal muscle, and the response of acetylcholine isdescribed as nicotinic response.

Requirements:

Animals : Frog

Drugs : Acetyl choline stock solution (1mg/ml)

Physiological solution: Frog Ringer

Procedure:

1. Pith or stun the frog and lay it on it back on the frog-dissecting board. Pin thefour limbs.
2. Remove the skin on the abdomen and expose the rectus abdominis muscle.
3. Cut and prepare two rectus muscle preparations from each frog. Tie a thread to the top and bottom of each muscle preparation before detaching the muscle from the body of the frog.
4. Mount the preparation in up-right position in the organ bath containing frog Ringer solution under a tension of 1 g. There is no need of maintaining the bathtemperature

since it is an amphibian tissue preparation. Bubble the organ with air.

5. Relax the tissue for 45 min. during which period wash the tissue with fresh quantum of ringer for at least four times.

6. Record the concentrations due to acetylcholine using either simple sideway or frontal writing lever. Ninety-second contact time and a total 5 min time cycle may be used for proper recording of the responses.

7. Record at least four responses to increasing doses of acetyl choline or till you get the maximum response. The maximum response is achieved if one gets sameor slightly less response with a higher concentration. Properly label the graph, put the date, your name, and fix the tracing with the help of fixing solution.

8. Measure the height of the response (mm) and draw a dose (concentration)-response graph.

Report:

Experiment No: 07

EFFECT OF PHYSOSTIGMINE ON DRC OF ACETYLCHOLINE USING FROG RECTUS ABDOMINIS MUSCLE AND RAT ILEUM RESPECTIVELY

Aim: To record the effect of Physostigmine (eserine) on the concentration-response curve of acetylcholine using rat ileum preparation.

Principle:

Rat colon is an intestinal smooth muscle. Ach causes the contraction of the muscleby acting on muscarinic receptor. The spontaneous contraction of muscle preparation can be reduced by calcium level of physiological salt solution and maintain the water bath at room temperature. The muscle preparation obtained from a starved rat gives a stable preparation. Physostigmine increased the levels of Ach.

Physostigmine is an anticholinesterase substance and it inhibits the metabolic break down of acetylcholine. As a result, the action of acetylcholine is potentiated.The concentration response curve of acetylcholine will be shifted to the left in thepresence of physostigmine.

Requirements:

Animal – Frog

Drugs – Acetylcholine stock solution (1 mg/ml), Physostigmine stock solution(1mg/ml)

Physiological solution – modified ringer with less calcium.

Procedure:

1. Step 1 to 6 are same as previous experiment
2. Record a concentration response curve of acetylcholine using at least four doses.
3. Add physostigmine (2 µg/ml) to the reservoir containing frog ringer and irrigatethe tissue with eserinised ringer 30 min.
4. Repeat the concentration-response curves of acetylcholine in the presence of physostigmine.
5. Label and fix both the concentration –response curves.
6. Plot both the concentration –response curves of acetylcholine, ie.one in the absence and the other in the presence of physostigmine. Note the potentiation in the response of acetylcholine.

Observation:

Report:

Experiment No: 08

Principle:

Rat colon is an intestinal smooth muscle. Acetylcholine causes the contractions of the smooth muscle by acting on muscarinic receptors. Atropine blocks muscarinic receptors in the smooth muscle. Therefore, atropine blocksacetylcholine-induced contractions in rat colon. The concentration-response curve of acetylcholine will be shifted to the right in the presence of atropine. Thenature of antagonism is of a competitive type.

The spontaneous contractions of the preparations can be reduced by reducing thecalcium content in the physiological solution and maintaining the bath at room temperature (23$\pm$ 2^0 C). The muscle (colon) preparation obtained from an un starved rat gives more stable contractions.

Requirements:

Animal : Rat (150-200g)

Drugs : Acetyl choline stock solution (1mg/ml),

Atropine stock solution (1 mg/ml) Physiologicalsolution :Modified Ringer (contains less calcium

Procedure:

1. Sacrifice the animal by cervical dislocation.

2. Cut open the abdomen and identify the colon. the right flexure, i.e., the subhepatic region of the colon where the ascending colon turns to become transverse colon, is cut out and placed in a shallow dish containing Modified Ringer's solution.

3. The lumen is gently cleaned and a 3 cm long tissue is mounted in the organ bath containing Modified Ringer's solution (pH 7.4) maintained at 25 ^{0} C and bubbled with carbonated air. The preparation is allowed to equilibrate for 45 minunder 500g tension.

4. Record the concentration dependent responses due to acetylcholine using frontal writing lever. Contact time 60 sec, and 5 min time cycle are kept for proper recording of the response.

5. Add atropine to the reservoir containing Modified Ringer's solution and irrigatethe tissue with atropinised Modified Ringer for 20 min.

6. Repeat the concentration-response curve of acetylcholine in presence of atropine.

7. Label and fix the tracing, and plot the graph as done in the earlier experiments.

8. Calculate EC 50 values and note the nature of antagonism.

Dose ratio = EC50 after atropine / EC50 before atropine

Inference:

Report:

Experiment No:09

BIOASSAY OF OXYTOCIN USING RAT UTERINE HORN BY INTERPOLATION METHOD.

Aim: To record the concentration –response curve of oxytocin using rat uterine hornby interpolation method.

Principle: Oxytocin is a hormone secreted by the posterior pituitary gland. The rat uterine preparation is commonly used for the bioassay of oxytocin. The sensitivity of the uterus to oxytocin depends on the estrous cycle. The various stages of estrous cycle can be identified by preparing vaginal smears and observing under microscope. Rat uterus is highly sensitive. An adult female rat (2-3 months old) has an estrous cycle of five days. The estrous cycle is divided into different stages.

1. Diestrus – characterized by presence of leukocytes in vaginal smear.

2. Proestrus /estrous – characterized by the presence of large number of nucleated epithelial cells.

3. Frank estrous –Presence of cornified epithelial cells.

4. Meta estrous or late estrous –presence of a mixture of nucleated, cornified epithelial cells and leucocytes.

If the rat is not in the frank estrous stage, it can be induced by the administrationof estrogen preparation, stilbestrol (0.1 mg/kg, sc:24 hrs. before)

Frank estrus uterus is highly sensitive to oxytocin and hence preferred forbioassay than the diestrus uterus which is relatively less sensitive.

Requirements:

Animal : Female rat (120-150 g)

Drugs : Oxytocin, Stilbestrol Physiological solution

De Jalon student organ bath

Procedure:

A. Preparation of animal:

1. Examine the vaginal smear under microscope to know about the proper stage of estrus cycle. If the rat is not in frank estrus, inject 0.1 mg/kg of stilbestrol and waitfor 24 hr. (Vaginal smear is prepared by taking a drop of vaginal wash and puttingon the glass slide).
2. If the epithelial cells are present in the smear, it is said to be in frank estrous phase.

B). Isolation of tissue:

1. Animal is sacrificed by cervical dislocation.
2. Cut open the pelvic region and expose both the horns of uterus. Separate them gently from the surrounding fatty material and transfer them into a dish containingDe Jalon's solution. When the rat is in estrus generally the uterus is fleshy and pink in color.
3. Then the uterus is cut longitudinally and a tissue portion of 2-3 cm long is taken and both ends are tied with the thread.

C) Mounting the tissue:

1. About 2-3 cm long tissue is mounted in organ bath containing De Jalon's solutionat 32^0C along with proper aeration.
2. A tension of about 500 mg (0.5g) is applied and tissue is allowed to equilibratefor 45 min.

D) Recording of the response:

1. Record the DRC for the standard oxytocin solution is taken.
2. Record responses due to 0.1,0.2, or 0.4 ml of the test substance. See that these responses would fall on the linear portion of the concentration –Response curve for the stand solution.
3. Label and fix the tracing.
4. Plot the concentration-response curve due to standard acetylcholine solution. Measure the heights of the contractions (response) due to different doses (A and B) of test solution. read the corresponding concentration from the standard curve.

Inference:

Report:

Experiment No: 10

BIOASSAY OF SEROTONIN USING RAT FUNDUS STRIP BY THREE POINT BIOASSAY

Aim: To find out the concentration of the given sample of 5 HT (5- hydroxtryptamine) or serotonin by three-point bioassay using rat fundus strip preparation.

Principle:

Rat fundus is a very sensitive tissue for the study of the action of several naturallyoccurring substances like 5-hydroxy tryptamine, histamine, acetyl choline and bradykinin. Unlike the intestinal smooth muscle(ileum) this preparation is slow contracting and slow relaxing type. Rat fundus is generally employed for the bioassay of serotonin. The fundus (the upper part of the stomach) is grey in colorand therefore, easily identified from pyloric part (pink in color). A zig-zag preparation of the fundal strip is prepared so as to expose maximum portion of the tissue to drug.

The tissue is sensitive to 1 ng/ml of serotonin,0.05-1 ng/ml of histamine and 0.2-

0.5 ng/ml of acetyl choline, respectively.

Principle of Three-point bioassay: It is a method based on the assumption of dose-response relationship. Log dose response curve is plotted and the dose of the standard producing the same response as produced by the test sample is directly read from the graph so to estimate the potency of the test sample.

In three-point bioassay, the DRC of standard, test samples are first obtained fromthe responses due to graded doses. From the DRC of standard, two standard dosesare selected in such a way that they have produced 20% and80 % of the maximalresponse respectively and are designated as S1 and S2. The responses of these doses lie on the steepest and straightest part (linear) of the curve. From The DRCof test sample one test dose is selected such that it gives a response which lies inbetween the two standard responses i.e., it gives a greater response than S1 and a

smaller response than S2 and is designated as T.

After selecting the standard and test doses, the bioassay is performed by recording the standard and test responses in a randomized fashion. The pattern ofaddition of doses is S1 S2 T; S2, T, S1 and T, S1, S2 in 3 successive cycles. Themean values of height of the contraction for all the 3 doses are calculated and areused in plotting the graph so as to estimate the potency of the test sample.

Advantages:

➢ More precision

➢ Reliability.

Requirements:

Animal:Rats (150-200 g, overnightfasted)

Drug :Serotonin (Stock solution10 µg/ml)

Krebs Solution

Procedure:

1. Sacrifice the rat by a blow on the head and carotid bleeding. Cut open the abdomen and expose the stomach.
2. Identify the fundus of the stomach (upper part). Incise it from the junction of pyloric part and put it in the dish containing Krebs solution.
3. Incise the fundus from the lesser curvature and open it longitudinally. Give alternate zig zag cuts to make a fundal stip preparation. Tie both the ends with thethread and mount in the organ bath containing Krebs solution at 37^0 C. Aerate thetissue.
4. Apply 1 gm load and allow the preparation to equilibrate for 30 mins. Using frontal writing lever with 10-12 magnification record the contractions due to increase concentrations of serotonin. Since the muscle contracts slowly and relaxes slowly,a contact time of 90 sec, and 5 min time cycle.
5. Select two doses form the DRC of standard drug, eliciting sub-maximal responses and bearing a dose ratio 1:2 preferentially and designate them as S1 and S2 andrespectively.
6. Select one dose from the DRC of test solution in such a way that the response dueto this dose lies preferentially between S1 and S2 and designate it as T.
7. Record 3 sets of responses due to S1 S2 and T adding them to organ bath in a randomized fashion as per the Latin square design mentioned in the principle. TheLatin square design of addition of doses is followed to ensure good randomizationand to account

8. for the fluctuating sensitivity of the tissue.
9. Measure various response to calculate the mean of each response (S1, S2, T)
10. Plot the graph with log dose on X-Axis and % of response on Y –axis and interpolate the T response onto the DRC of standard in between S1 and S2 so as to find the standard dose that gives an equivalent response of that of test.
11. Calculate the potency of the test drug by converting the log of the standard dose that has produced an equivalent response as that of test into anti-log and report the potency as a number of µ gms/ml.
12. Concentration of unknown = $\frac{n1/t \times \text{anti-log}\{T\text{-}S\ 1 \times \log n2/n1\}\ Cs}{S2\text{-}S1}$

Where, n1 = lower standard dose (n2) = Higher standard dose
(t)= test dose
S1= response of n1 S2 = response of n2 T = response of tCs=
Concentration of standard

Report:

The potency of the test drug, serotonin is estimated as ---------- µgms/ml bythree-point method of bioassay.

Experiment No: 11

TO STUDY THE ANTI-INFLAMMATORY PROPERTY OF INDOMETHACIN AGAINST CARRAGEENAN-INDUCED ACUTEPAW OEDEMA IN RATS

Aim: To study the anti-inflammatory property of indomethacin against carrageenan induced acute paw oedema in rats.

Principle: Inflammation is a tissue –reaction to infection, irritation or foreign substance. It is a part of the host defense mechanism but when it becomes great itis a hopeless condition. Aging is also considered to be an inflammatory response.There are several tissue factors or mechanisms that are known to be involved in the inflammatory reactions such as release of histamine, bradykinin and prostaglandins.

This method is based upon the ability of anti-inflammatory agents to inhibit the edema produced in the hind paw of the rat after injection of a phlogistic agent (carrageenan). The volume of the injected paw is measured before and after the application of irritants. The paw volume of treated animals is compared with control. A plethysmograph is used to measure paw volume.

Requirements:

Animal: Rats (150-200g)

Equipment: Plethysmograph (simple apparatus containing mercury. The mercury displacement due to dipping of the paw can be directly read from scale attached to the mercury column or adjusting the mercury level in the arm B to theoriginal level by moving the arm B up/down and note the volume required in boththe arms equal), syringe and needle

Drugs: carrageenan (1% w/v solution and inject 0.1 ml underneath the plantar region) Indomethacin (Dose 20 mg/kg, s.c, Prepare a stock solution containing 4mg/ml of the drug and inject 0.5 ml/100 g of body weight of the animal). Saline(0.9%)

Procedure:

1. Weigh the animals and number them.
2. Mark a mark on both the hind paws (right and left) just beyond tibio-tarsal junction, so that every time the paw is dipped in the mercury column upto the fixedmark to ensure constant paw volume.
3. Note the initial paw volume (both right and left) of each rat by mercury displacement method.

4. Divide the animals into two groups each comprising of at least four rats. To one group inject saline and to the second group inject indomethacin subcutaneously.

5. After 30 min inject 0.1 ml of 1% (w/v) carrageenan in the plantar region of the left paw of control as well as indomethacin –treated group. The right paw will serve as reference non-inflamed paw for comparison.

6. Note the paw volume of both legs of control and indomethacin-treated rats at 15,30,60, and 120 min after carrageenan challenge.

7. Calculate the percent difference in the right and left paw volumes of each animalof control and indomethacin –treated group. Compare the mean percent change in paw volume in control and drug–treated animals and express as per cent edemainhibition by the drug.

Observations:

Report:

Experiment No: 12

TO STUDY THE ANALGESIC EFFECT OF MORPHINE IN MICE USING HOT PLATE METHOD

Aim: To study the Analgesic effect of given drug **(**morphine) in mice using hot plate method (Eddy and Leimbach)

Principle: Pain is an unpleasant feeling, which make us uncomfortable and reduce the physical as well as mental alertness. Although pain is also useful for usbecause it acts as a warning signal and it warns about something uncommon insideor outside our body. If the pain is minor, it may be tolerated but if the pain becomes severe it has to be managed at earliest.

Analgesia is defined as a state of reduced awareness to pain, and analgesics are substances that decrease pain sensation (pain–killers) by increasing the thresholdto painful stimuli. The commonly used analgesics are aspirin, paracetamol (non- narcotic type) and morphine (narcotic type).

Painful reactions in experimental animals can be applying noxious (unpleasant) stimuli such as

(i) Thermal (radiant heat as a source of pain)

(ii) Chemical (irritants such as acetic acid and bradykinin)

(iii) Physical pressure (tail compression)

in the laboratory commonly used procedures are tail-flick (tail-withdrawal from the radiant heat) method using analgesiometer, hot plate (jumping from the hot plate at 55ºC) method and acetic acid –induced writhing.

Requirements:

Animal: Mice (20-25 g)

Equipment : Eddy's hot plate Morphine sulphate (dose 5 mg/kg, sc, prepare a stock solution containing 0.5 mg/ml and inject 1 ml/100 g obody weight of mouse)

Procedure:

1. Weigh and number the mice.
2. Take the basal reaction –time by observing hind paw licking or jump response (whichever appears first) in animals when placed on the hot plate maintained at constant temperature (55º C). normally animals show such response in 6-8 sec. Acut off period of 15 sec is observed to avoid damage to the paws.

3. Inject morphine to animals and note the reaction time of animals on the hot plateat 15, 30, 60 and 120 min after the drug administration. As the reaction time increases with morphine,15 sec is taken as maximum analgesia and the animals are removed from the hot plate to avoid injury to the paws.

4. Calculate percent increase in reaction time (as index of analgesia) at each time interval.

Observation:

Report:

Experiment No: 13

TO STUDY THE ANALGESIC EFFECT OF MORPHINE IN MICE USING TAIL FLICK METHOD

Aim: To study the Analgesic effect of given drug **(**morphine) in mice using Tailflick method.

Principle: Pain is an unpleasant feeling, which make us uncomfortable andreduce the physical as well as mental alertness. Although pain is also useful for us because

it acts as a warning signal and it warns about something uncommon inside or outside our body. If the pain is minor, it may be tolerated but if the pain becomessevere it has to be managed at earliest.

Analgesia is defined as a state of reduced awareness to pain, and analgesics are substances which decrease pain sensation (pain –killers) by increasing threshold to painful stimuli. The commonly used analgesics are aspirin, paracetamol (non- narcotic type) and morphine (narcotic type).

Painful reaction in experimental animals can be applying noxious (unpleasant) stimuli such as

(i) Thermal (radiant heat as a source of pain)

(ii) Chemical (irritants such as acetic acid and bradykinin)

(iii) Physical pressure (tail compression)

In the laboratory commonly used procedures are the tail-flick (tail-withdrawal from the radiant heat) method using analgesia meter, hot plate (jumping from thehot plate at 55º C) method, and acetic acid–induced writhing.

Requirements:

Animals: Mice (20-25 g)

Equipment: Analgesiometer

Drugs: Morphine sulphate (dose 5 mg/kg, sc. Prepare a stock solution containing 0.5 mg/ml and inject 1 ml/100 g of body weight of mouse)

Procedure:

1. Weigh and number the mice.

2. Take basal reaction time to radiant heat by placing the tip (last 1-2 cm) of the tailon the radiant heat source. The tail withdrawal from the heat (flicking response) is taken as the endpoint. Normally, a mouse withdraws its tail within 3-5 sec. A cut-off period of 10-12 sec is observed to prevent damage to the tail. Any animalfailing to withdraw its tail in 3-

5 sec is rejected from the study.

3. Take at least 3-5 basal reaction times for each mouse at a gap of 5 min. to confirmnormal behavior of the animal.

4. Inject morphine and note the reaction time at 5,15,30,60 min after the drug. As the reaction time reaches 10 sec it is considered maximum analgesia and the tail is removed from the source of heat to avoid tissue damage.

5. Calculate the percentage increase in reaction time (index of analgesia) at each time interval.

Observation:

Report:

Experiment No: 14

TO STUDY THE ANALGESIC EFFECT OF MORPHINE AGAINST ACID- INDUCING WRITHING IN MICE

Aim: To study the Analgesic effect of morphine against acid-inducing writhingin mice.

Principle: Painful reactions in animals may be produced by chemicals also. Intraperitoneal injection of phenyl quinone, bradykinin or acetic acid produces pain reaction which is characterized as a writhing response.

Constriction of the abdomen, turning of trunk (twist) and extension of hind legs are taken as reaction to chemically induced pain. Analgesics, both narcoticand non-narcotic type, inhibit writhing response.

Requirements Animals:

Mice (25-30 g)

Drugs: Morphine sulphate (dose 5 mg/kg, sc. Prepare a stock solution containing 0.5 mg/ml and inject 1 ml/100 g of body weight of mouse), acetic acid 1%v/vInject 1 ml/100 g of body weight of the animal.

Procedure:

1. Weigh and number the mice.

2. Divide the animals into two groups, each consisting of 5 animals. Administer appropriate volume of acetic acid solution to the first group (which serves as control), place them individually under glass jar for observation.

3. Note the onset on wriths. Record the number of abdominal contractions, trunk twist response, and extension of hind limbs as well as the number of animals showing such response during a period of 10 min.

4. To the second group of animals inject morphine. Fifteen minutes later, administeracetic acid solution to these animals. Note the onset and severity of writhing response as done in step 3.

5. Calculate the mean writhing scores in control and morphine-treated groups. Note the inhibition of pain response by morphine.

Inference:

Report:

Vision and Mission of the Institution

Vision

The East Point College of Pharmacy aspires to be a globally acclaimed institution, **recognized** for **excellence in** pharmaceutical education, research and nurturing students for **holistic development.**

Mission

M1 Create pharmacy graduates through **quality education**

M2 Promote innovation, **creativity**, and excellence **in teaching**, learning, and **research**

M3 **Inspire** integrity, teamwork, critical thinking**, personal** development, and ethics in **students** and lay **the** foundation for lifelong learning

M4 **Serve** the **healthcare, technological, scientific**, and **economic** needs of then **society**.