AmbujaNeotia

THE NEOTIA UNIVERSITY

ज्ञानम् आत्म प्रदीपाय

V – SEMESTER (III B.Pharm)

PHARMACOLOGY- II

PRACTICAL LAB MANUAL

PHARMACOLOGY-II

EX NO:1

Introduction to *In-vitro* pharmacology and physiological salt solutions.

Aim: To study the Introduction about In-Vitro Pharmacology and Physiological saltsolutions.

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 petridishes) 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 vivo environments. Artificial conditions are formed by mixing the necessary components andreagents under controlled conditions inside a glassware in the laboratory.

In vitro methods are widely used in Pharmaceutical industry to produce large scale pharamaceuticals 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 detailed analysis

In vitro studies replacing studies in whole animals.It is

less expensive and provides quicker results.

Definition:

Estimation of potency of an active ingredient in unit quantity of preparation is detection and measurement of concentration of substance preparation using biologicalmethod 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 produce samebiological 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 predominantor 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 of drugs are 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 match is response with dose of standard is found by trial and error.From this the potency of unknowntest 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 tracked response on the dose axis.

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 1 st later 2nd and 3rd response of the unknown which fall in between linear portion of 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 taken into 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 should be linearportion of the concentration curve and also the ration between the dose should be perfectly1:2 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 be recorded and magnified.

a.Fulcrum

b.Stylus

Magnification:

The fulcrum should be placed that there is some magnification of the actual concentration.In order to achieve this distance between the writing point and the fylcrum 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.syringes

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 as PSS /Ringer solutionIts 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 metabolilsm.

Commonly used PSS

1. **Frog ringer**- (For heart,rectus abdominis and other prepartions 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 fundud strip,Tracheal preparations, Vas deferens)etc

The PSS should:

It Maintains tissue outside the animal body .

Select PSS in which tissue last longer.

Prepare the solution with the help of distilled or double distilled or deionised water. Prepare fresh solution.

Ingredients	Concentration in gms/l			
	Frog ringer	Tyrode	Kreb'sbicarbonate	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 ontractility of the prepartion
2.	Potassium Chloride (KCl)	To maintain ionic balance
3.	Calcium Chloride($CaCl_2$)	To maintain the contracttility 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 prepartion and hence to reduce the spontaneous activity

Report-

EX NO:2

Effect of drugs on isolated frog heart

Aim: To study the effect of drugs(inotropic and chronotropic actions) on perfused frog 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 parasympathomimetics such as acetylcholine produce negative inotropic and negative chronotropic response.

Requirements:

Animal :frog

Apparatus : frog's ringer solution,reservoir,tubing,screw clip,syme,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 ringer

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 and expose 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 bottle containing frog ringer.Insert a cannula in the vein and tie the thread to assure the cannula in place.

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

6. Adjust a proper venous pressure of 2-4 cm by altering the height of perfusion bottle.The effective venous pressure is the height in cms from level of the venous cannula and the ringer level in the perfusion bottle.Use of marriott's bottle helps in attaining the constant pressure. Start the perfusion by opening screw clamp attached to the tube.

7.Pass a thin pin hook through the tip of the ventricle,and with the help of a fine thread attached to the hook,tie it to the free limb of the universal lever,which is fixed to a stand.Adjust proper tension and magnification by altering the height of the lever.Record the normal contraction of the heart on the

smoked drum.

8.Inject 0.1, 0.2, 0.5 and 1 ml of the stock solution of each drug in a sequential order and note the change in the rate and amplitude of contraction. Keep at least 5 min 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 venous cannula. Take precautions to avoid any leakage of the drug from the tube, and the injection of air bubbles.

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

Observation:

Report:

EX NO:3

EFFECT OF DRUGS ON NORMAL AND HYPODYNAMIC PERFUSED FROG HEART

AIM: To study the effect of drugs on normal and hypodynamic frog heart.

Principle: The myocardial contraction of normal and hypodynamyic frog heart takes place according to starling's law of heart. According to this law force of systolic contraction is directly proportional to the fibre length in diastole. Since systolic contraction represents cardiac output and the fibre lenth in diastole indicates venous pressure, the law indicates that caradiac 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 systolic contraction.When the heart is in this state,i.e. inability to contract to physiological normal it is said to be 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 (stock solution 100 µg/ml)

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

Procedure:

1.Set up the perfusion of frog heart with normal frog ringer solution as describes in the 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 ¼ th the Calcium chloride as compared to that of normal ringer. Note the change in the pattern of 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 $CaCl_2$ (0.1,0.2,0.4 and 0.5 ml) .Note the change in contractility. 5.Fix the tracing and compare the responses of these drugs in normal and hypodynamic heart.

Observation:

Report:

EX NO:4

Effect of drugs on blood pressure and heart rate of dog

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 is through the vagus supply the heart. Parasympathetic innervations iss through the vagus whereas the sympathetic nerve supply to the heart comes from fibres arising from stellate or inferior cervical ganglion. The nervous supply to the blood vessels aare 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. Drugs which increase the blood pressure are called pressor agents, and those decrease are called.

Requirements:

Animal –Dog (6-8 kg)

Anaesthetic pentobarbitone sodium (45 mg /kg ,iv ;prepare a stock solution contiaining45mg/ml of the drug and per kg of body weight.

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

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

Procedure:

1. Anaesthetise 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 mercury manometer.

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

5.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. Wait for 5-10 min.

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

Inference:

Report

EX NO:5

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. Normal urine 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,hudroflumethiziade and frusemide. Increase in volume of urine is measured with the help of measuring cylinder and compared 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)

Frusemide(5mg/kg;oral)

Procedure:

1. albino rats (150-200 g) are fasted (deprived of food and water) overnight andsaline(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 (N.Saline)

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

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

(iv) IVth group-saline +frusemide(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 noted and thevolume is recorded at intervals of 15 min for 3-4 hrs.

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

Observation:

Report:

EX NO:6

DRC of acetylcholine using frog rectus abdominis muscle

Aim: To record a concentration response curve of acetylcholine using rectus abdominismuscle 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 in the dose orthe concentration of the drug.The dose –response curve is sigmoid or S- shaped.The first part(25% of graph) of the curve has poor discrimination between the doses.whereas the middleportioin of the curve shows greater sensitivity to different concentrations,and the responses toincreasing concentrations are linearly differentiated. The last of the curve (plateau)shows theceiling effect where no more increase in the response is seen with further increase in the dose.

When the doses are increased in geometric progression (logarithimic intervals) and the response is plotted against logarithms of doses,the relationship is called log dose-responsecurve. Rectus abdominis muscle is a skeletal muscle,and the response of acetylcholine is describedas 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 its back on the frog-dissecting board.Pin the four 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 andbottom of each muscle preparation before detaching the muscle from the body of the frog.

4.Mount the preparation in up-rght position in the organ bath containing frog Ringer solution under a tension of 1 g. There is no need of maintaining the bath temperature 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 same or slightly less response with a higher concentrations.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

EX NO:7

Effect of physostigmine on DRC of acetylcholine using frog rectusabdominis 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 cause the contraction of the muscle by acting on muscarinic receptor.The spontaneous contraction of muscle preparation can be reduced by calcium level of physiologoical 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 concentrationresponse curve of acetylcholine will be shifted to the left in the presence of physostigmine.

Requirements:

Animal –frog

Drugs –Acetlycholine 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 irrigate the tissuewith 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:

EX NO:8

Effect of atropine on DRC of acetylcholine using frog rectus abdominis muscle and rat ileum respectively.

Aim: To record the concentration-response curve of acetylcholine and its modification byatropine using rat ileum preparation.

Priniple:

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 blocks acetylcholine induced contractions in rat colon. The concentration response curve of acetylcholine will be shifted to the right in the presence of atropine. the nature of antagonism is of competitive type .

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

Requirements:

Animal :Rat (150-200g)

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

Physiological solution: 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 regionof 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 carbogenated air. The preparation is allowed to equilibrate for 45 min under 500g tension. 4. Record the concentration dependent responses due to acetylcholine using frontal writinglever. 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 irrigate the tissuewith atropinised Modified Ringer for 20 min.
6. Repeat the concentration-response curve of acetylcholine in presence of atropine. 7. Label and fix the tracing ,plot the graph as done in the earlier experiments. Calculate EC 50values and note the nature of antagonism

$$\text{Dose ratio} = \frac{\text{EC 50 after atropine}}{\text{EC 50 before atropine}}$$

Inference:

Report:

EX NO:9

Bioassay of oxytocin using rat uterine horn by interpolation method.

Aim: To record the concentration –response curve of oxytocin using rat uterine horn byinterpolation 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 oestrous cycle.The various stages of oestrous 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 oestrous cycle of five days.The oestrous cycle is divided into different stages.

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

2.Proestrous /estrous – charactereised by the presence of large number of nucleated epithelial cells.

3.Frank oestrous –Presence of cornified epithelial cells.

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

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

Frank oestrus uterus is highly sensitive to oxytocin and hence preferred for bioassay than the dioestrous 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. <u>preparation of animal</u>:

1. Examine the vaginal smear 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 stilbestrol and wait for 24 hr.(Vaginal smear is prepared by taking a drop of vaginal wash and putting on the glass slide).

2. If the epithelial cells are present in the smear ,it is said to be in frankoestrous phase.

B). <u>Isolation of tissue:</u>

1.Animal is sacrificied 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 containing De jalon's solution. when the rat is in oestrus generally the uterus is fleshy and pink in colour.

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 jalons solution at 32^0C along with proper aeration.

2. A tension of about 500 mg (0.5g)is applied and tissue is allowed to equilibrate for 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 concentratin –Response curve for the stand solution

3. Label and fix the tracing.

4. Plot the concentration response curve due to standard acetyl choline 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:

EX 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-hydroxy tryptamine) orserotonin 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 naturally occurring 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 gernerallyemployed for the bioassay of serotonin.The fundus (theupper part of the stomach) is grey in colour and therefore ,easily indentified from pyloric part (pink in colour).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 acety 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 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 20% and80 % of he maximal response 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 DRC of test sample one test dose is selected such that it gives a response which lies in between 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 of addition of doses is S1 S2T; S2 ,T, S1 and T, S1, S2 in 3 successive cycles.The mean values of height of the 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.

Advantages:

More precision
Reliability.

Requirements:

Animal : Rats (150-200 g ,overnight fasted) Drug
: serotonin (Stock solution 10 μg/ml)
Physiological solution : Krebs solution

Procedure :

1. Sacrifice the rat by a blow on the head and carotid bleeding.2.Cut open the abdomen and expose the stomach.
3.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.
4.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 the thread and mount in the organ bath containg krebs solution at 37^0 C .Aerate the tissue.
5.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 ,and5 min time cycle.

6. Select two doses form the DRC of standard drug,eliciting sub-maximal responses andbearing a dose ratio 1:2 preferentially and designate them as S1 and S2 and respectively. 7.Select one dose from the DRC of test solution in such a way that the response due to thisdose lies preferentially between S1 and S2 and designate it as T.

8.Record 3 sets of responses due to S1 S2 and T adding them to organ bath in a randomized fashion as per Latin square design mentioned in the princilple.The latin square design of addition of doses is followed to ensure good randomization and to account 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 on to 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 in to anti-log and report the potency as number of µ gms/ml.

12.Concentration of unknown = $n1/t \times \text{anti log}\{T\text{-}S\ 1 \times \log n2/n1\} Cs$

$$\frac{\text{------}}{S2\text{-}S1}$$

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

Report:

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

EX .NO:11

Determination of PA2 value of prazosin using rat anococcygeus muscle (by Schilds plot method)

Aim: To determine the pA2 value of prazosin using rat anococcygeus muscle.

Principle:

The pA2 value is devised by Sir Heinz Otto Schild in Quantitative Pharmacology.In 1947 pA scale ,to express drug antagonism.

pA_X value is calculated to compare the potency of antagonists acting on the same receptor.

The pA_X value is defined as the negative logarithm of the molar concentration of the antagonist required to reduce the effect of a multiple dose(x) of the agonist to that of a single dose in the absence of antagonist. The meaning of **pA2** is the affinity of the antagonist to the receptor.For antagonist ,efficacy is 0 and affinity is one.

Higher the pA_X value,more potent is the antagonist. The determination of pA2 (X=2) and pA10 (X=10) values have wider applications.If the difference between these two values is found to be 0.95 or very near,the antagonism is likely to be of competitive type.an antagonist acting on the same receptor will have same pA2 value in all the tissue or organ preparations.

Principle of schlids plot:

- calculation of pA2 value for an antagonist from the effects observed on isolated smooth muscles can be done in two-ways: using schild's plot procedure or schild'sequation.
- most commonly used method for estimating pA2 value.
- The Schild plot is a pharmacological method of receptor classification.
- Plot log (dose ratio-1) against negative log molar concentration of the antagonist(B) used (or directly against B)
- When the slope of the line so obtained is unity ,then the antagonism is competitive.

Requirements:

Animal: Rat (150-200 g,overnight fasted)

Drugs : Prazosin stock solution

EX NO:12

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

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

Principle: Inflammation is a tissue –reaction to infection,irritation or foreign substance.It is a part of the host defence mechanism but when it becomes great it is 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 (carragennan).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.Plethysmograph is usedto 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 the original level by movingthe arm B up/down and note the volume required in both the 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 thatevery time the paw is dipped in the mercury column upto the fixed mark to ensure constant paw volume.

3. Note the initial paw volume (both right and left) of each rat by mercury displacementmethod.

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

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

6. note the paw volume of both legs of control and indomethacin-treated rats at 15,30,60,and120 min after carragenan challenge.

7. Calculate the percent difference in the right and left paw volumes of each animal of controland indomethacin –treated group. Compare the mean percent change in paw colume in control and drug – treated animals and express as per cent oedema inhibition by the drug.

Observations:

Report:

EX NO:13

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 hotplatemethod.(Eddy and Leimbach)

Principle: pain is an unpleasant feeling, which make us uncomfortable and reduce the physical as well as mental alertness. Although pain are also useful for us because it acts as awarning 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 becomes severe 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 (narcotictype).

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 tail-flick (tail-withdrawal from the radiant heat) method using analgesiometer,hot plate (jumping from the hot plate at 55 ° C) method and acetic acid –induced writhing.

Requirements:

Animal:Mice (20-25 g)

Equipment:Eddy's hot plate

Drugs: Morphine sulphate (dose 5 mg/kg,sc.,prepare a stock solution containing 0.5 mg/mland inject 1 ml/100 g of body 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 (55° C).normally animals show such response in 6-8 sec. A cut 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 plate at 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

EX NO:14

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 usingTail flick method.

Principle: pain is an unpleasant feeling, which make us uncomfortable and reduce the physical as well as mental alertness. Although pain are also useful for us because it acts as awarning 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 becomes severe 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 (narcotictype).

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 tail-flick (tail-withdrawal from the radiant heat)method using analgesiometer,hot plate (jumping from the hot plate at 55 ° C) methodand 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/mland 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 tail on the radiant heat source.The tail-withdrawal from the heat (flicking response) is taken as the end point.Normallu 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 animal failing to withdraw its tail in 3-5 sec is rejected from the study.Take at least 3-5 basal reaction times for each mouse at a gap of 5minto confirm normal behaviour of the animal.
3. Inject morphine and note the reaction time at 5,15,30,60 min after the drug.As the reactiontime reaches 10 sec it is considered maximum analgesia and the tail is removed from the source of heat to avoid tissue damage.
4. Calculate percentage increase in reaction time (index of analgesia) at each time interval.

Observation:

Report

EX NO:15

To study the Analgesic effect of morphine against acid-inducing writhing in mice

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

Principle: Painful reactions in animals may be produced by chemicals also. Intraperitoneal injection of phenylquinone, bradykinin or acetic acid produces pain reaction which is characterised as a writhing response. Constriction of abdomen, turning of trunk (twist) and extension of hind legs are taken as reaction to chemically induced pain. Analgesics, both narcotic and 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/v Inject 1 ml/100 g of bodyweight of the animal.

Procedure:

1. Weigh and number the mice.

2. Divide the animals into two groups, each consisting of 5 animals.Administer appropriatevolume 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 writhes,.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, administer acetic 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: