TECHNOCRATS

Lab Work Book of

Pharmacology-III

(BP - 608 P)

Department of Pharmacy

Lab Manual of

Pharmacology-III

(BP - 608 P)

Pric e :

Edition :

Disclaimer
Every possible effort has been made to bring out this book accurately to fullfill aspirations of all readers. The publisher and their associates do not make any warranty with respect to accuracy, completeness of the book and hence can not be held liable in any way for the loss or damage whatsoever.

Printed & Published by :

TECHNOCRATS
PUBLICATIONS

Arera Colony, Bhopal.
e-mail : technocratspublications@gmail.com

TECHNOCRATS
PUBLICATIONS

Lab Work Book
of

Pharmacology-III

(BP-608 P)

(Strictly According to RGPV Syllabus)

Name : .....

Enrollment No. : .....

Institute : .....

Academic Session : .....

Department of Pharmacy

TECHNOCRATS
PUBLICATIONS

Vision of the Institute

To grow as an institute of Excellence for Pharmacy Education and Research and to serve the humanity by sowing the seeds of intellectual, cultural, ethical, and humane sensitivities in the students to develop a scientific temper, and to promote professional and technological expertise.

Mission of the Institute

M 1: To inculcate ethical, moral, cultural and professional values in students

M 2: To provide state of art infrastructure facilities to the staff and students so as to enable them to learn latest technological advancements

M 3: State of art learning of professionalism by the faculty and students

M 4: To produce well learned, devoted and proficient pharmacists

M 5: To make the students competent to meet the professional challenges of future

M 6: To develop entrepreneurship qualities and abilities in the students

PROGRAM OUTCOMES (POs)

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

PEOs

PEO 1: To inculcate quality pharmacy education and training through innovative Teaching Learning Process.

PEO 2: To promote professionalism, team spirit, social and ethical commitment with effective interpersonal communication skills to boost leadership role assisting improvement in healthcare sector.

PEO 3: To enhance Industry-Institute-Interaction for industry oriented education and research, which will overcome healthcare problems of the society.

PEO 4: To adapt and implement best practices in the profession by enrichment of knowledge and skills in research and critical thinking

PEO 5: To generate potential knowledge pools with interpersonal and collaborative skills to identify, assess and formulate problems and execute the solution in closely related pharmaceutical industries and to nurture striving desire in students for higher education and career growth.

Course Outcomes (COs):

Student will be able to:

- CO1: Determination of PA2 value of antagonists on various isolated tissue.
- CO2: Interpretation of unknown concentration of drugs by various bioassay methods.
- CO3: Identify the antiulcer activity of a drug using pylorus ligation method in rats.
- CO4: Determination of PA2 value of agonists on various isolated tissue.
- CO5: Interpret various drug actions on experimental animals.

Index

Experiment No.1

AIM:

Anti- allergic activity by mast cell Stabilization assay.

REFERENCE:

Prussin C, Metcalfe DD. IgE, Mast cells, Basophils and Eosinophils. J Allergy Clin Immunol 2003; 111(2):486-94.

Choudhary GP. Mast Cell Stabillizing Activity of Piper longum Linn. Indian J Allergy Asthma Immunol. 2006; 20:112-6.

REQUIREMENT:

Animals: Albino rats of 175-200 g of either sex

Drugs: Disodium chromoglycate (50mg/Kg s.c.)

Reagents: Saline solution (0.9%), RPMI 1640 buffer medium (pH 7.2-7.4), Egg albumin (100 g/ml), Toludine blue solution (1%)

Instruments: Microscope with 10X magnification lens

THEORY:

In allergic diseases mast cells plays an important role by defending the antigens. IgE antibodies formed in response to antigen antibody complex attaches to the surface receptors of mast cells and rises calcium influx leading to degranulation of mast cell which releases some pro-inflammatory mediators (also known as local hormones) such as histamine and eicosanoids.

Aim of this experiment is to screen anti-allergic activity of the drugs.

PROCEDURE:

Mast cell stabilization activity

Albino rats of either sex are divided into two groups consisting of 3 animals in each. Group-I receives normal saline, and Group -2 receives Disodium chromoglycate (50 mg/kg, i.p,) for 3 days. Inject 10 ml/kg of 0.9 % saline into peritoneal cavity on 4^{th} day to each animal. Message the peritoneal region of the animal gently for 5 min, then collect the peritoneal fluid and transfer to the test tube which is carrying 7-10 ml of RPMI buffer. Centrifuge the fluid for 400-500 RPM .Discard the supernatant and wash the pellets of mast cells twice with same buffer by centrifugation. Add egg albumin to the above cell suspension and incubate at 37 for 10 min. Later the suspension has to stain with 1 % toluidine blue solution and observe the slide under microscope for calculating number of granulated and degranulated mast cells in each group (total 100 cells are has to be counted from different visual areas).

OBSERVATIONS

Group	Total number of cells (n=100)	
	Granulated	Degranulated
Group-1 (saline)		
Group-2 Disodium chromaglycate (50mg/kg I.P.)		

RESULTS AND DISCUSSION:

VIVA QUESTIONS

Q.-1. What is mast cell degranulation?

.....

.....

.....

.....

Q.-2. Write physiological effects of histamine?

.....

.....

.....

.....

Q.-3. Explain in brief synthesis and release of histamine?

.....

.....

.....

.....

Experiment No. 2

AIM:

Study of anti-ulcer activity of a drug using pylorus ligand (SHAY) rat model.

REFERENCE:

Shay H. A. Simple method for the uniform production of gastric ulceration in the rat. Gastroenterol .1945; 5:43-61.

Vinothapooshan G, Sundar K. Anti-ulcer activity of Mimosa pudica leaves against gastric ulcer in rats. RIPBCS, 2010; (4); 606-14.

REQUIREMENTS:

Animals: Albino Wistar rats of 150-200 g are selected for the study.

Drugs: Ether (anaesthetic), Ranitidine 20 mg/kg p.o, 0.9 % normal saline

Reagents: 0.1 N NaOH, Phenolphthalein, Topfer's reagent,

Instruments: Dissecting microscope (10 % magnification lens), Burette pH meter, Surgical instruments.

THEORY:

Peptic ulcer is one of the most prevalent gastrointestinal disorders. The aim of the present study is to demonstrate the antiulcer activity of drugs using pylorus ligand (SHAY) rat model. This was first demonstrated by Shay in 1945. Ligation of rat pylorus results gastric acid accumulation in the fore-stomach leads to acute gastric ulcers. This procedure is used to screen the drugs for their anti-secretary and antiulcer activity.

PROCEDURE:

Animals are to be divided into two groups consisting of 3 animals in each group. Saline is to be administered to control group and Ranitidine (20 mg/kg P.O.) to other group. Animals have to be faced for one day with free access to water. 30 min prior to ligation process, the drug (Ranitidine) should be given. Under light ether anaesthesia a midline abdominal incision is made and pylorus will be legated with proper care and the wound is closed. Then rats are to be placed individually in separate cages without food and water during this period and allowed them to recover. Sacrifice the animals by decapitation after 4 hours and open the stomach and collect the stomach contents in a centrifuge tube. Determine the pH pHmeter. Open the greater curvature of the stomach and clean the part with saline. Fewer than 10 % magnification lens ulcers are to be observed and ulcer index is calculated by using the formula given below.

Ulcer index =

$(U1+U2+U3) \times 10^{-1}$

U1= Average of number of ulcers per animal

U2= Average of severity score

U3= Percentage (%) of animals with ulcer

Intensity of ulcers with scoring 0- normal coloration, 0.5-red coloration; 1- spot ulcer, 1.5 – hemorrhagic stress; 2- deep ulcer; 3- perforations.

Ulcer score 1 mm (exact) =1; 1-2mm=2;>2 mm =3;>3mm=4

Analysis of stomach contents

Measure total volume of gastric content and centrifuge at 1000 rpm for 10 minutes. Pipette out one ml of supernatant liquid of the centrifuged content and dilute with 10 ml distilled water. Titrate the liquid against 0.01N NaOH using Topfer's reagent as indicator, till the end point (appearance of orange colour). The volume of NaOH used is to be noted to estimate free acidity. Titration has to be continued till the appearance of pink colour and the volume of NaOH run down is to be noted to calculate total acidity.

Calculation of acidity is given below:

OBSERVATIONS

Group	Volume of gastric contents (in ml)	pH	Free acidity (mEq/l)	Total acidity (mEq/l)	Ulcer index
Control (saline)					
Standard (Ranitidine 20 mg/kg P.O.)					

RESULTS AND DISCUSSION:

.....

.....

.....

.....

.....

.....

.....

.....

.....

.....

VIVA QUESTIONS

Q.-1. Explain in brief pathophysiology of ulcer formation

.....

.....

.....

.....

Q.-2. Write mechanism of proton pump inhibitors.

.....

.....

.....

.....

Q.-3. Discuss role of prostaglandin in cytoprotectin.

.....

.....

.....

.....

Experiment No. 3

AIM:

Study of anti- ulcer activity of a drug using NSAIDS.

REFERENCE:

Wallace JL. Baillieres Best Pract Res Clin Gastroenterol, 2000; 14(1): 147-59.

Rao ChV et al Antiulcer activity of Utleria salicifolia rhizome extract. J Ethanopharmacol 2004; 91: 243-9.

REQUIREMENTS:

Animals: Albino Wistar rats of (200-300 g) either sex

Drugs: Aspirin -400 mg/kg, p.o., Ranitidine 30 mg/kg p.o., Dissolve both drugs in 1% CMC.

Chemicals: 1% CMC

Instruments: Dissecting microscope (10 X magnification lens), Surgical instruments.

THEORY:

Peptic ulcer defined as an ulceration of the mucous membrane of the stomach, duodenum or oesophagus. The imbalanced secretions of gastric acid, pepsin and duodenal mucosal defence mechanisms are the causes of such ulcers. Excessive non-steroidal anti-inflammatory drugs (NSAIDs) consumption of can cause damage of gastrointestinal mucosa leading to ulcers. Aim of this experiment is to screen the drugs for their antiulcer activity.

PROCEDURE:

Select albino rats weighing 200-300 g and divide them into two groups consisting of 3 animals in each group. Group-1 receives 1% CMC; Group -2 receives Ranitidine (30mg/kg, p.o.). Administer Aspirin (200 mg/kg)/ Diclofenac sodium (100 mg/kg) suspended in 3 ml of CMC after 30 minutes of antiulcer drug administration in both groups. Avoid access to feed and water to animals. After 6 hours sacrifice the animals by cervical decapitation, open along the greater curvature of the stomach, remove the stomach contents and wash with 0.9 % saline. Observe for the ulcers formed and measure the length of each ulcer and calculate ulcer index.

Ulcer score: 1 mm (pin point) =1; 1-2 mm=2; >2 mm=3; >3 mm=4

Ulcer index = $(U1+U2+U3) \times 10^{-1}$

U1= Average of number of ulcers per animal

U2= Average of severity score

U3= Percentage (%) of animals with ulcer

Intensity of ulcers with scoring: 0- normal coloration,0.5-red coloration, 1-spot ulcer, 1.5-hemorrhagic stress,2-deep ulcer and 3-perforations.

OBSERVATIONS

Group	Ulcer index
Control (saline + NSAIDs)	
Standard (NSAIDs + Ranitidine 20 mg/kg P.O.)	

RESULTS AND DISCUSSION:

VIVA QUESTIONS

Q.-1. Explain in brief physiology of gastric acid secretion?

.....

.....

.....

.....

Q.-2. How antihistaminic are effective in gastric ulceration?

.....

.....

.....

.....

Q.-3. How H. pylori cause gastric ulcer?

.....

.....

.....

.....

Experiment No. 4

AIM:

To study the effect of drugs on gastrointestinal motility.

REFERENCE:

Furness JB, Enteric nervous system. Scholarpedia. 2007; 2(10): 4064.

Peddireddy MKR. In vitro Evaluation Techniques for Gastrointestinal Motility. Indian J Pharmaceutical Education Res. 2011; 45 (2):184-91.

REQUIREMENTS:

Animals: Medium sized rabbit

Drugs : Adrenaline/ Acetylcholine – 10 ug/ml, Atropine sulphate – 100 ug/ ml, isoproterenol / isoprenaline 10 ug/ ml, Propranolol – 1 mg / ml, phenylephrine – 10 ug/ml, phentolamine – 0.1 ug/ml

Solutions: Tyrode solution

Apparatus used : Kymograph, Dissecting board, Dissecting board, Disecting instruments, scissors, petriplates, Syringe, Frontal writing lever, water bath with temperature controlling unit, organ bath with aeration tube

THEORY:

Intestinal motility is regulated by the enteric nervous system of the gut (Auerbachs and Meissners plexuses) and the activity of this system can be modified by autonomic nervous system. Hence effect of sympathomimetic and parasympathomimetic drugs on intenstinal motility can be studied by using isolated piece of intestine. Parasympathomimetic drugs stimulate enteric neurons to release acetylcholine at neuromuscular junctions and enhance muscle tone and rhythmicity of intestine. Sympathomimetic drugs act on alpha and beta receptors and release adrenaline which in turn prevents release of acetylcholine and inhibits muscle tone and rhythmicity.

Many animal models can be employed to study intestinal motility of sympathetic and parasympathetic drugs. Guinea pig ileum is advantageous for assay purposes as it produces as it produces steady baseline for studying effects of drugs. Rabbit intestine (ileum, duodenum, jejunum) usually jejunum is used for the effects of pendulum movements (continuous contraction and relaxation-Finklman method). In the present study rabbit ileum is selected for estimating the effects of selected drugs on intestinal motility.

PROCEDURE:

The procedure adopted for the study is the modified.

Finkleman method developed by Walker and Scott. Select a medium sized rabbit for the study. Fast the animal for 24 hours prior to experiment as food in gut results in messy dissection and flushing of gut contents may damage the intestine. Before sacrificing the rabbit, prepare Tyrode Ringer solution and place

about 250 ml of this solution in an ice cold flask. Sacrifice the animal by cervical decapitation without using use of antiseptic as it may affect the gut motility. Shave the abdomen of the animal and vacuum the surface to remove adhered fur. Make a midline incision through the skin and abdominal muscles. Locate ileum and a part of ileum was taken 10 cm away from ileocaecal valve. An optimal length of tissue (5-6 cm) is cut carefully and ties the thread to antimesenteric border on both sides and place them in the Tyrode solution (extra pieces of ileum can be stored in ice cold Tyrode solution so that they are viable for hours. In ice cold solution the motility will ceases but after placing them in warm solution the tissue gets relaxed and shows motility within 5-10 minutes).

Record the rhythmic activity of the ileum by using frontal writing lever and kymograph. Suspend the tissue in organ bath of Tyrode solution (100 ml) at 37 with adequate oxygen supply (mixture of 95 % O_2 and 5 % of Co_2). Tie one end of the thread of the tissue to fixed point inside the organ bath and the other end to the lever for recording contractions on the kymograph. Stabilise the tissue in the solution to the conditions for about 30 minutes. Ensure the lever should be placed horizontally and record the normal contractions followed by effects of drugs on muscles.

After recording normal contraction inject the drugs one by one and observe the force of contraction and tone (normal increased or decreased) frequency of contractions (per minute) before and after drug administration.

Inject 0.1 ml of drugs in the succession order in the organ bath and the responses are recorded. After noting the effect of every drug, drain the muscle bath and refill with fresh warm Tyrode solution (100 ml) . Take the control (without drug) reading before and after each drug response. Maintain wash out period for 15-20 minutes for change of every drug and check the next drug response only the when the tone and amplitude returned to original value approximately. The drug and done name should be mentioned in the recording after taking response of each drug.

Order of adding drugs

0.1 ml of Ach- 1.0 ml of Ach-0.1 ml of NE (these drugs are postganglionic neurotransmitters of parasympathetic and sympathetic system. Ach increases contraction and NE relaxes the tissue)

1.0 ml of phenylephrine- 1.0 ml of isoproterenol (phenylephrine causes contraction by inhibiting adenylate cyclise – alpha adrenergic agonists; isoproterenol causes relaxation by showing beta agonist action)

1.0 ml of phenatolamine (alpha adrenergic blocker). Wait for 2 minutes then proceed for adding 1.0 ml of phenylephrine (to check for alpha receptor blockade, if they are blocked, if they are blocked no response is seen)

1.0 ml of propranolol (beta adrenergic blocker). Wait for 2 minutes then proceed for adding 1.0 ml of isoproterenol (to check for alpha receptor blockade, if they are blocked no response is seen)

1.0 ml of atropine – wait for 3 minutes – add 1.0 ml of Ach-(to check for parasympatholytic activity of atropine)

OBSERVATION

RESULTS AND DISCUSSION:

VIVA QUESTIONS

Q.-1. Give composition of Tyrode solution?

.....

.....

.....

.....

Q.-2. Write pharmacological effects of acetylcholine?

.....

.....

.....

.....

Q.-3. Write note on enteric nervous system (ENS)?

.....

.....

.....

.....

Experiment No. 5

AIM:

Effect of saline purgative on frog intestine.

REFERENCE:

Laxative. Available at: http:// www.britannica.com/science/laxative#ref800128.

REQUIREMENTS:

Animal: frog

Reagents: 0.9% to 0.45 % of saline (hypotonic), 27 % Magnesium sulphate (hypertonic), Frogs Ringer solution (isotonic)

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

THEORY:

Saline purgatives are the salts comprising of highly charged ions and do not crosses cell membrane freely. They remain inside the lumen and retain water through osmotic forces. They increase the volume of the content of the bowl. Stretch the colon and produces normal stimulus for contraction of the muscle that leads to defecation. The aim of the present study is to examine the effect of saline purgative on frog intestine.

PROCEDURE:

Path the frog and place it on a dissecting board. Expose the abdominal cavity and carefully trace the small intestine. Make the small intestine into three compartments by tying threads of different colours in such a way that no fluid can move from one compartment to the other. Inject 0.2 ml of each hypotonic solution into first compartment. 0.2 ml of hypertonic solution to second compartment and 0.2 ml of isotonic solution into third compartment. Wait for 20 minutes and the observations are to be record.

OBSERVATION

Drug	Compartment	Effect
Hypotonic solution (0.2ml of 0.9 % of saline)	First compartment	
Hypertonic solution (0.2 ml of 27 % magnesium sulphate)	Second compartment	
Isotonic solution (0.2 ml of frogs Ringer solution)	Third compartment	

RESULTS AND DISCUSSION:

VIVA QUESTIONS

Q.-1. Give composition of frog ringer solution?

.....

.....

.....

.....

Q.-2. Write in brief about osmotic purgatives?

.....

.....

.....

.....

Q.-3. Discuss various uses of purgatives?

.....

.....

.....

.....

Experiment No. 6

AIM:

Insulin hypoglycaemic effect in rabbit.

REFERENCE:

Lubert S. Biochemistry. Fourth edition. New York: W H Freeman and Company; 1995:773-774.

Goyal RK. Principles and Methods of Bioassay, Ahmedabad. 2008: 14-16.

REQUIREMENTS:

Animals requirement : Healthy rabbits weighing 1800-3000 gms.

Drugs: 20 units of insulin preparation. One unit contains 0.4082 mg of insulin

Reagents : Normal saline, HCl, 0.5% phenol, 1.4-1.8% glycerine.

THEORY:

Insulin is a peptide hormone produced by the beta cells of pancreas to high glucose levels in the blood. Released insulin acts on the insulin receptors on body cells and activities glucose transporters to absorb more glucose into the cells thereby regulates carbohydrate, protein and fat metabolism in body cells. Reduced blood glucose levels inhibit insulin release and stimulate alpha cells of pancreas to release glucagon to maintain glucose levels in the blood by gluconeogenesis. The aim of the present study is to evaluate the effect of insulin in rabbits at different time intervals.

PROCEDURE:

Select healthy rabbits weighting 1800-3000 gms for the study. They should be maintained in uniform diet for 7 days. Fast the animals for 18 hrs with no access to water before starting the procedure. Select three animals for the study and inject 1 unit/ml of insulin.

Prepare drug solution freshly. Weigh 20 units of insulin accurately and dissolve it in normal saline. Acidify the solution by using HCL to pH 2.5. Add 0.5 % of phenol as preservative and 1.4-1.8 % of glycerine and make the final volume to 20 units/ml of solution.

Withdraw 2 ml of blood from marginal ear vein of each rabbit and estimate blood glucose level by using suitable biochemical method and the concentration of glucose can be noted down as initial blood glucose level. Then inject insulin (1 unit/ml) to the animals and check the blood sugar level up to 5 hours at the interval of 1 hour each and the determine blood glucose levels as final blood sugar level and compared both initial and final blood glucose levels.

OBSERVATION

Animals	Initial blood sugar level (in mg/ml)	Final blood glucose level at different time intervals (in mg/ ml) in ours				
		1	2	3	4	5
1st animal						
2nd animal						
3rd animal						
Average mean value						

RESULTS AND DISCUSSION:

VIVA QUESTIONS

Q.-1. Explain in brief physiological actions of insulin?

.....

.....

.....

.....

Q.-2. Discuss role of insulin in diabetes mellitus?

.....

.....

.....

.....

Q.-3. Write mechanism of action of sulfonylureas?

.....

.....

.....

.....

Experiment No. 7

AIM:

To study pyrogen testing in rabbits.

REFERENCE:

Jayanthi VR. Pyrogens. Available at: http://www.pharmainfo.net/pyrogens.

Sawadadkar NP, Thakur T. Pyrogen testing. Available at: http://www.slideshare.net/nilesh1208/pyrogen-testing.

REQUIREMENTS:

Animals: Healthy matured rabbits of either sex

Instruments used: Pyrogen free syringes, needles, glass ware, clinical thermometer or thermistore proble

Reagents: 0.9 % sodium chloride injection as diluents

THEORY:

Pyrogens are the fever inducing organic substances (metabolic products of microorganisms) responsible for many febrile reactions. Pyrogen testing should be done to every batch of pharmaceutical product (particularly parenterals) for which water is the usual vehicle. The best animal model for pyrogen test is the rabbit as it generals reproducible results that are similar to threshold response to humans and also economic.

PROCEDURE:

Select healthy matured rabbits of either sex for the study. House them individually in the place that is free from disturbances that likely to excite them and maintain the room temperature at 20-23 . Make all the materials and equipment pyrogen free either by heating the same at 250 for not les than 30 minutes or any other method. Standardized clinical thermometer with precision of 0.1 is used to measure rectal temperature of the rabbit. Test them to determine that maximum reading is attaining in <5 minutes or not.

Pyrogens testing of solutions should done in two steps

A. Preliminary test (Sham test)

Conduct Sham test for animals using for first time in pyrogen testing or have not been used during the two previous weeks. Hence acclimatize them for 1-3 days before using for pyrogen testing of sample. Select three rabbits and fast the rabbits overnight with free access to water and withhold water during the test. Inject sterile pyrogen free saline solution intravenously at a dose of 10 ml per kg of body weight. Record the temperature of animals 90 minutes prior to the injection and continue for 3 hours at an interval of half an hour after injection. Exclude any animal showing temperature variation of 0.6 or more for main test.

B. Main test

Select three rabbits that passed the Sham test. Determine the initial body temperature of the rabbits and it should be between 38-39.8. Dilute Solution to test with pyrogen free saline solution or any solution prescribed in monograph. Warm the test liquid to 38.5 before injection. Inject the test solution to the animal slowly into the marginal vein of the ear for a period of not more than 4 minutes and the volume injected should not less than 0.5 ml/kg and not more than 10 ml/kg of body mass. Determine the temperature of each animal at every half an hour for 3 hours after injection.

INTERPRETATION OF THE RESULT

Consider any decrease in final body temperature as rise in zero. Consider the test solution as pyrogen free if the response in individual rabbit showing a temperature less than 0.6 or if the sum of responses of three rabbits does not exceed 1.4. If the temperature exceeds than the above, then continue the test using other five rabbits. If not more than 3 rabbits out of eight shows individual rise in body temperature of 0.6 or if the sum of the responses of the group of 8 rabbits does not exceed 3.7 then the preparation under test is passed and considers it as pyrogen free.

OBSERVATION

Table 1: Recording of initial and final temperatures

No. Of rabbits	**Initial body temp. (in)**	**Final body temperature (in)**					
		30 min	60 min	120min	180min	240min	300min
1st animal							
2nd animal							
3rd animal							

Table 2: Result of pyrogen test

No. Of Rabbits	**Individual body temperature raise (in)**	**Body temperature rise in group (in)**	**Test**
1st animal			Pass /fail
2nd animal			
3rd animal			

RESULTS AND DISCUSSION

VIVA QUESTIONS

Q.-1. What are non-selective COX inhibitors?

.....

.....

.....

.....

Q.-2. How NSAIDs increase gastric acid secretion?

.....

.....

.....

.....

Q.-3. Write note on acute paracetamol poisoning?

.....

.....

.....

.....

Experiment No. 8

AIM:

To study the diuretic activity of drugs

REFFERENCE:

Jayasree T, Kiran KK. Evaluation of the diuretic effect of the chloroform extract of the Benincasa hispida rind extract in guinea pigs. J Clini Diagnos Res.2011;5(3):578-82.

REQUIREMENTS:

Animals: Make Albino Wistar rats of 140-200 g of either sex.

Drugs: 2% CMC in normal saline- 10 ml/kg, Furosemide- 10 mg/kg p.o. in vehicle.

Instruments: pH meter, volumetric tubes.

THEORY:

Diuretics are the drugs that increase urine output and help in treating mild to moderate hypertension by reducing volume of the plasma and thus venous return to the heart. The aim of the study was to screen the diuretic activity of given test drug.

PROCEDURE:

Divide selected male Albino Wistar rats into two groups consisting of 3 animals in each. Group-1 receives 2% CMC in normal saline and serves as control and the other group receives Furosemide and serves as test group. After treatment immediately hydrate the rats with 15 ml/kg of saline and place them in metabolic cages separately and maintain the room temperature to 21 0.5 and avoid access to feed and water. Care to be taken to avoid mixing of urine and faeces. After 5 hours measure the total volume of urine collected in measuring cylinder and compares the same in two groups. Other parameters like urine pH and concentration of electrolytes such as sodium, chloride and potassium are to be estimated by using ion selective electrode method described y the manuals in biochemical kits.

OBSERVATION

Group	Parameters				
	Urine volume (in ml)	Urine pH	Concentration of sodium (m.mol/L)	Concentration of potassium (m.mol/L)	Concentration of chloride (m.mol/L)
Group -1 (2% cmc in normal saline)					
Group-2 (Furosemide-10 mg/kg, p.o.)					

RESULTS AND DISCUSSION

VIVA QUESTIONS

Q.-1. Discuss mechanism of action of Furosamide?

.....

.....

.....

.....

Q.-2. What is the role of Carbonic anhydrase enzyme in urine physiology?

.....

.....

.....

.....

Q.-3. What are osmotic diuretics?

.....

.....

.....

.....

Experiment No. 9

AIM:

To study of biotransformation and excretion of drug.

REFFERENCE:

Kang MJ et al. Pharmacologically Active Metabolite of Currently Marketed Drugs: Potential Resources for New Drug Discovery and Development. Pharmaceutical Soc. Japan.2010; 130:1325-37.

REQUIREMENTS:

Drugs: Aspirin (1 gm)

Reagents: Ferric chloride (1%)

THEORY:

Biotransformation is defined as a process in which organic compounds are converted to other forms by the action of bacteria, fungi and enzymes. This process helps in determination of pharmacokinetic properties of compounds and also plays a very important role in toxicity estimations. Ecretion is a process by which a drug is eliminated from an organism either in metabolite from or in uncharged form.

Aspirin/ acetylsalicylic acid is metabolized in the liver to form salicylic uric acid and are mainly excreted by kidneys through urine.

This study was conducted with the objective to understand the metabolism and excretion of parent compound.

PROCEDURE

Select three volunteers for the study. Request them void their bladders and to collect the urine samples. Label those samples as control. Later give 1 gm of aspirin orally with 250-400 ml of water to the volunteers. Ask them to collect urine samples for 3 hours for every 30 minutes. Take 3 ml of urine from each sample and add a drop of 1 % ferric chloride. Appearance of violet colour indicates the presence of salicylic group of salicylic acid. Mark positive test with sign (+) in the observation table.

OBSERVATION

Time at which urine sample is collected	Presence of salicyl groups in volunteers		
	I	II	III
30 min			
60 min			
90 min			
120 min			
150 min			
180 min			

RESULTS AND DISCUSSION

VIVA QUESTIONS

Q.-1. Define enzyme induction and inhibition?

.....

.....

.....

.....

Q.-2. What are the factors affecting metabolism of drug?

.....

.....

.....

.....

Q.-3. What are the factors affecting drug excretion?

.....

.....

.....

.....

Experiment No. 10

AIM:

To record the concentration response curve of histamine and its modification by an anti histaminic using guinea pig ileum preparation.

REFFERENCE:

"Hand Book of Experimental Pharmacology" by S.K. Kulkarni, Ninth Edition 2007, Published by Vallabh Prakashan, New Delhi, Page No. 92-94.

REQUIREMENTS:

Animal: Guinea pig 400-600 g, overnight fasted

Drugs: Histamine stock solution 1mg/ml, mepyramine stock solution 1mg/ml

Physiological salt solution: Tyrode

THEORY:

Histamine is an autocoid having profound physiological effect in the body. Beside the triple response caused by it, histamine has spasmogenic response on intestinal smooth muscle. By acting on H1-histaminic receptors it causes the contraction of intestinal smooth muscle. Guinea pig is highly sensitive to histamine and guinea pig ileum preparation is very commonly used for isolated tissue work. Mepyramine is a selective H1-histamine receptor antagonist.

PROCEDURE:

1. The guinea pig is sacrificed by a blow on the head and carotid bleeding.
2. Cut open the abdomen and lift the caecum to trace the ileocaecal junction. Cuyt & remove a few cm. long of the ileac portion and immediately place it in the watch glass containing Tyrode solution. Trim the messentary and with gentle care clean the contents of the ileum by pushing the tyrode solution into the lumen of the ileum utmost care should be taken to avoid any damage to the gut muscle. Cut the ileum into small segments of 3 cm long.
3. Take 1 piece of the ileum of 2-3 cm and tie the thread to top and trhe bottom ends without closing the lumen and mount the tissue in the organ bath containing tyrode solution maintained at 32'c-35'c and bubbled with oxygen air. A tension of 0.5g is created or applied and the tissue is allowed to equilibrate for 30 minutes before adding drugs to the organ bath.
4. Record the concentration dependent responses due to histamine using frontal writing lever. Contact time of 30 sec' 5 min time cycle are kept for proper recording of the responses.
5. Record at least 4 concentration dependent responses due to histamine.
6. Add mepyramine to the reservoir containing tyrode solution and irrigate the tissue with mepyramine containing tyrode solution. For 30 min.

7. Repeat the concentration response curve of histamine in the presence of mepyramine.
8. Label and fix the tracing and plot as done in the earlier exp.
9. Calculate the EC50 and note the nature of antagonism.

$$\text{Dose ratio} = \frac{\text{EC50 after mepyramine}}{\text{EC50 before mepyramine}}$$

OBSERVATION

RESULTS AND DISCUSSION

VIVA QUESTIONS

Q.-1. Discuss in brief histaminic receptors?

.....

.....

.....

.....

Q.-2. Write note on guinea pig as experimental animal?

.....

.....

.....

.....

Q.-3. Give adverse effects of H1-anti histaminic?

.....

.....

.....

.....

Experiment No. 11

AIM:

To calculate pA2 value for atropine using acetylcholine as agonist employing guinea pig ileum preparations.

REFFERENCE:

"Hand Book of Experimental Pharmacology" by S.K. Kulkarni, Ninth Edition 2007, Published by Vallabh Prakashan, New Delhi, Page No. 95-97.

THEORY:

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

The pAx 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. Higher the pAx value, more potent is 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.

REQUIREMENTS:

Animal: Guinea pig 400-600 g, overnight fasted

Drugs: Acetylcholine stock solution 1mg/ml, Atropine stock solution 1mg/ml

Physiological salt solution: Tyrode

PROCEDURE:

1. The guinea pig is sacrificed by a blow on the head and carotid bleeding.
2. Cut open the abdomen and lift the caecum to trace the ileocaecal junction. Cuyt & remove a few cm. long of the ileac portion and immediately place it in the watch glass containing Tyrode solution. Trim the messentary and with gentle care clean the contents of the ileum by pushing the tyrode solution into the lumen of the ileum utmost care should be taken to avoid any damage to the gut muscle. Cut the ileum into small segments of 3 cm long.
3. Take 1 piece of the ileum of 2-3 cm and tie the thread to top and trhe bottom ends without closing the lumen and mount the tissue in the organ bath containing tyrode solution maintained at 32'c-35'c and bubbled with oxygen air. A tension of 0.5g is created or applied and the tissue is allowed to equilibrate for 30 minutes before adding drugs to the organ bath..
4. Record concentration dependent concentrations due to acetylcholine until a peak response is obtained.
5. Select two dose bearing 1:2 ratio and elucidating sub maximal responses (A, 2A) for pA2 determination.

6. Standardise the tissue with the selected doses of acetylcholine a tissue is said to be standardize when it responds identically to the same dose of an antagonist wehen repeated.
7. Record the conc. Due to the double doses of ach. (2A) in the presence of varying conc. (B1, B2, B3,) of atropine.
8. Consider the response due to double dose of Ach i.e before adding atropine as 100% response.
9. Plot a graph representing negative log of the molar conc. Of an atropine employed along x-axis and % responses along y-axis.
10. Read out the pA2 value for atropine from the graph directly. It corresponds to the % responses obtained with half the dose of ach.

OBSERVATION

pA2 values of Atropine and Mepyramine in different tissue preparations

Tissue	Atropine- acetylcholine	Mepyramine- histamine
Guinea-pig ileum		
Guinea-pig trachea		
Guinea-pig lung (perfused)		

RESULT AND DISCUSSION

.....

.....

.....

.....

.....

.....

.....

.....

.....

.....

.....

.....

VIVA QUESTIONS

Q.-1. What is concentration response curve?

.....

.....

.....

.....

Q.-2. Give physiological effects of acetylcholine?

.....

.....

.....

.....

Q.-3. Write note on choline esterase inhibitors

.....

.....

.....

.....