SCHEME : J

Name : _____

Roll No. : _____ Year : 20___ 20___

Exam Seat No. : _____

LABORATORY MANUAL FOR

PHARMACOLOGY (20056)

SECOND YEAR D.PHARMACY

MAHARASHTRA STATE BOARD OF TECHNICAL EDUCATION, MUMBAI
(Autonomous) (ISO 9001: 2015) (ISO/IEC 27001:2013)

VISION

To ensure that the Diploma level Technical Education constantly matches the latest requirements of Technology and industry and includes the all-round personal development of students including social concerns and to become globally competitive, technology led organization.

MISSION

To provide high quality technical and managerial manpower, information and consultancy services to the industry and community to enable the industry and community to face the challenging technological & environmental challenges.

QUALITY POLICY

We, at MSBTE are committed to offer the best in class academic services to the students and institutes to enhance the delight of industry and society. This will be achieved through continual improvement in management practices adopted in the process of curriculum design, development, implementation, evaluation and monitoring system along with adequate faculty development programmes.

CORE VALUES

MSBTE believes in the following

* Skill development in line with industry requirements
* Industry readiness and improved employability of Diploma holders
* Synergistic relationship with industry
* Collective and Cooperative development of all stake holders
* Technological interventions in societal development
* Access to uniform quality technical education

A LABORATORY MANUAL
OF
PHARMACOLOGY
(20056)

Second Year
Diploma in Pharmacy (PH)

Maharashtra State
Board of Technical Education, Mumbai.
(Autonomous)
(ISO 9001:2015) (ISO/IEC27001:2013)

PCI ER- 2020 / 'J' Scheme Curriculum

Maharashtra State Board of Technical Education, Mumbai
(Autonomous)(ISO: 9001:2015)(ISO/IEC 27001:2013)
4th Floor, Government Polytechnic Building, 49, Kherwadi,
Bandra (East), Mumbai- 400051,
(Printed on July, 2024)

MAHARASHTRA STATE BOARD OF TECHNICAL EDUCATION, MUMBAI

CERTIFICATE

This is to certify that, Mr. /Ms. _____

Roll No. _____ of Second Year Diploma in Pharmacy studying at (Institute)_____

has completed the practical work satisfactorily in Pharmacology (20056) for the academic year 20 - 20 as prescribed in the PCI ER 2020 syllabus.

Date: _____ Enrollment No. : _____

Place: _____ Exam Seat No. : _____

Course Teacher **Principal**

External Examiner

Seal of the Institute

MAHARASHTRA STATE BOARD OF TECHNICAL EDUCATION MUMBAI

PROGRAM OUTCOMES

1. **Pharmacy Knowledge:** Possess knowledge and comprehension of the core and basic knowledge associated with the profession of pharmacy.
2. **Modern tool usage:** Learn, select, and apply appropriate methods and procedures, resources, and modern pharmacy-related computing tools with an understanding of the limitations.
3. **Leadership skills:** Understand and consider the human reaction to change, motivation issues, leadership and team-building when planning changes required for fulfilment of practice, professional and societal responsibilities. Assume participatory roles as responsible citizens or leadership roles when appropriate to facilitate improvement in health and wellbeing.
4. **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).
5. **Pharmaceutical Ethics:** Honour 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.
6. **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.
7. **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.
8. **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.
9. **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 on-going basis.

COMPETENCIES FOR THE INDIAN D.PHARM HOLDERS

Competency is defined as "A distinct composite of knowledge, skill, attitude and value that is essential to the practice of the profession in real life contexts".

The candidates who successfully complete the Diploma in Pharmacy (D. Pharm) program of Education Regulations 2020 (ER-2020), from the institutions approved by the Pharmacy Council of India are expected to attain the following professional competencies.

1. Review Prescriptions: The student should receive and handle prescriptions in a professional manner and be able to check for their completeness and correctness. Also, the prescribers should be contacted for any clarifications & corrections in the prescriptions with suggestions if any.

2. Dispense Prescription / Non-Prescription Medicines: The student should be able to dispense the various scheduled drugs / medicines as per the implications of the Drug & Cosmetic Act and Rules there under. Also, the non-prescription medicines (over-the-counter drugs) should be dispensed judicially to the patients as required.

3. Provide Patient Counselling / Education: The student should be able to effectively counsel / educate the patients / caretakers about the prescription / non-prescription medicines and other health related issues. Effective communication includes using both oral and written communication skills and various communication techniques.

4. Hospital and Community Pharmacy Management: The student be able to manage the drug distribution system as per the policies and guidelines of the hospital pharmacy, good community pharmacy practice and the recommendations of regulatory agencies. Also, be able to manage the procurement, inventory, and distribution of medicines in hospital / community pharmacy settings.

5. Expertise on Medications: The student should be able to provide an expert opinion on medications to health care professionals on safe and effective medication – use, relevant policies and procedures based on available evidences.

6. Proficiency on Pharmaceutical Formulations: The student should be able to describe the chemistry, characteristics, types, merits and demerits of both drugs and excipients used in pharmaceutical formulations based on her/his knowledge and scientific resources.

7. Entrepreneurship and Leadership: The student should be able to acquire the entrepreneurial skills in the dynamic professional environments. Also, be able to achieve leadership skills through teamwork and sound decision-making skills.

8. Deliver Primary and Preventive Healthcare: The student should be able to contribute to various healthcare programs of the nation including disease prevention initiatives to improve public health. Also contribute to the promotion of national health policies.

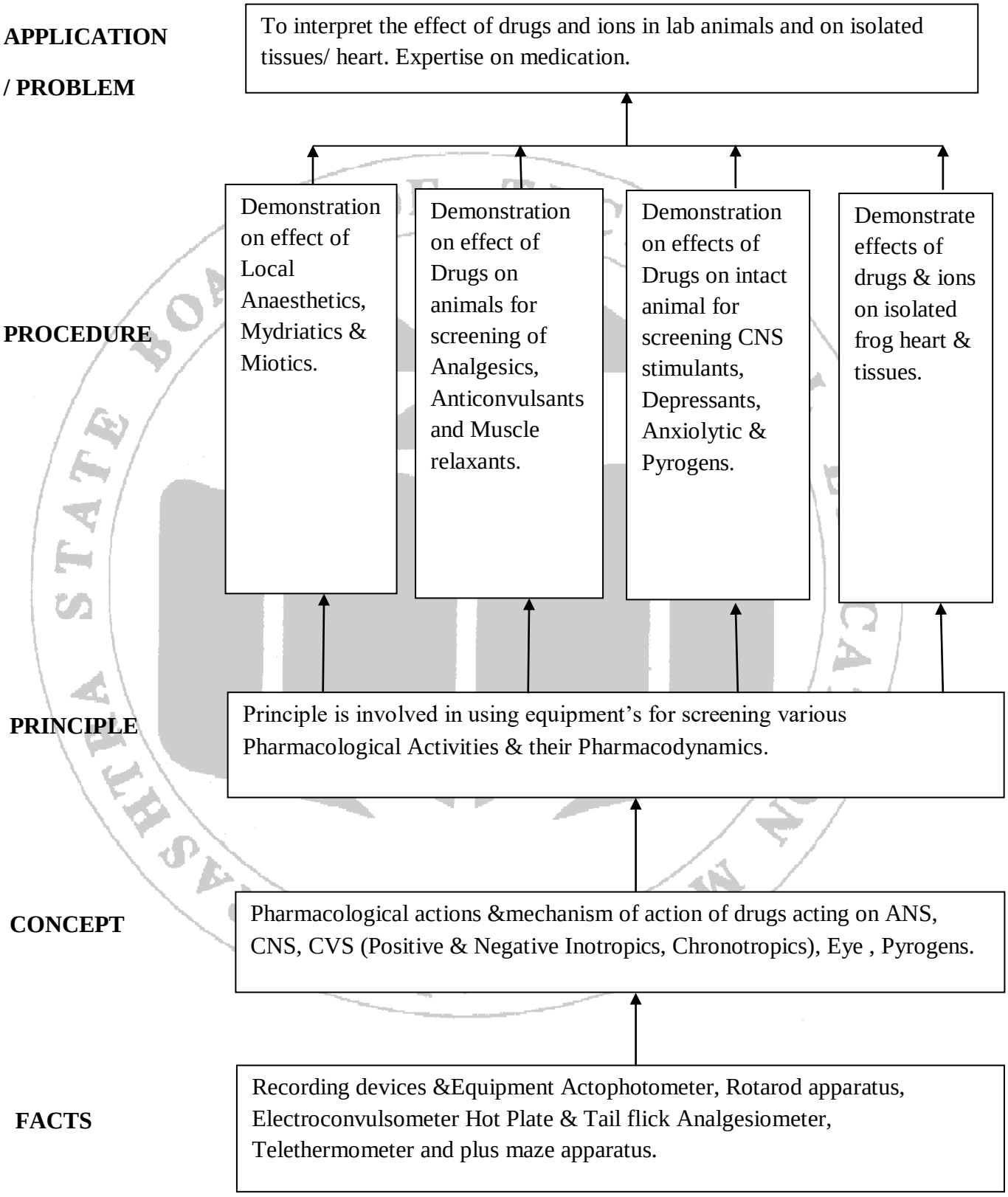
9. Professional, Ethical and Legal Practice: The student should be able to deliver professional services in accordance with legal, ethical and professional guidelines with integrity.

10. Continuing Professional Development: The student should be able to recognize the gaps in the knowledge and skills in the effective delivery of professional services from time to time and be self-motivated to bridge such gaps by attending continuing professional development programs.

COMPETENCY MAPPING WITH THE COURSE

Competencies	Pharmacology
1.Review Prescriptions	✓
2.Dispense Prescription / Non-Prescription Medicines	✓
3.Provide Patient Counselling / Education	✓
4.Hospital and Community Pharmacy Management	
5.Expertise on Medications	✓
6.Proficiency on Pharmaceutical Formulations	✓
7.Entrepreneurship and Leadership	
8.Deliver Primary and Preventive Healthcare	✓
9.Professional, Ethical and Legal Practice	
10.Continuing Professional Development	✓

GRAPHICAL STRUCTURE OF SUBJECT AREA
PHARMACOLOGY (20056)



PHARMACOLOGY – PRACTICAL

Course Code: ER20-21P **50 Hours (2 Hours/week)**

Scope: This course provides the basic understanding about the uses, mechanisms of actions, dose dependent responses of drugs in simulated virtual animal models and experimental conditions.

Course Objectives: This course will demonstrate / provide hands-on experience in the virtual platform using appropriate software on the following

1. Study of pharmacological effects of drugs like local anaesthetics, mydriatic and mitotic on rabbit eye
2. Screening the effects of various drugs acting in the central nervous system
3. Study of drug effects on isolated organs / tissues
4. Study of pyrogen testing on rabbit

Course Outcomes: Upon successful completion of this course, the students will be able to

CO1. Study and report the local anesthetic, mydriatic and mitotic effects of the given drug on the rabbit eye

CO2. Choose appropriate animal experiment model to study the effects of the given drugs acting on the central nervous system and submit the report

CO3. Perform the effects of given tissues (simulated) on isolated organs / tissues and interpret the results

CO4. Interpret the dose dependent responses of drugs in various animal experiment models

Practical's Introduction to the following topics pertaining to the experimental pharmacology has to be discussed and documented in the practical manuals.

1. Introduction to experimental pharmacology
2. Study of laboratory animals (a) Mice; (b) Rats; (c) Guinea pigs; (d) Rabbits
3. Commonly used instruments in experimental pharmacology
4. Different routes of administration of drugs in animals
5. Types of pre-clinical experiments: In-Vivo, In-Vitro, Ex-Vivo, etc.
6. Techniques of blood collection from animals

Experiments Note: Animals shall not be used for doing / demonstrating any of the experiments given. The given experiments shall be carried- out / demonstrated as the case may be, ONLY with the use of software program(s) such as 'Ex Pharm' or any other suitable software

1. Study of local anaesthetics on rabbit eye
2. Study of Mydriatic effect on rabbit eye
3. Study of Miotic effect on rabbit eye
4. Effect of analgesics using Analgesiometer
5. Study of analgesic activity by writhing test
6. Screening of anti-convulsant using Electro Convulsiometer
7. Screening of Muscle relaxants using Rota-Rod apparatus
8. Screening of CNS stimulants and depressants using Actophotometer
9. Study of anxiolytic activity using elevated plus maze method
10. Study of effect of drugs (any 2) on isolated heart
11. Effect of drugs on ciliary motility on frog's buccal cavity
12. Pyrogen testing by rabbit method

Assignments

The students shall be asked to submit written assignments on the following topics (One assignment per student per sessional period. i.e., a minimum of **THREE** assignments per student)

1. Introduction to Allergy Testing
2. Introduction to Toxicity Studies
3. Drug Facts Labels of US FDA
4. Pre-clinical studies in new drug development
5. Medicines and meals: Before or After food
6. Pre-clinical studies in new drug development
7. Drugs available as paediatric formulations
8. Drug information apps

Field Visit

No field visit mentioned in the syllabus for Pharmacology (20056)

STRATEGY FOR IMPLEMENTATION

It is suggested that 32-35% experiments (8-9 no's) and one assignment shall be completed before every sessional exam.

GUIDELINES FOR TEACHERS

Teacher shall explain the following points to the students before starting of the practical:

1. **Learning Objectives:** To foster better understanding of the subject and to inculcate the skills and attitude related practicals.
2. **Graphical structure:** In graphical structure topics and subtopics are organized in systematic way so that ultimate purpose of learning the subject is achieved. This is arranged in the form of fact, concept, principle, procedure, application and problem.
3. **Elementary Guide to work in Laboratory:** The methods and other finer details of the equipment including equipment specifications should be explained to avoid equipment breakages, create conducive environment for proper organizing of the practical work with the time schedule.
4. Teachers should verify and check the work conditions of the equipment and request the students to follow the standard operating procedures (SOP).
5. Before starting the practical, Teachers should explain the strategies of the experiment.
6. Mannequin, simulated cases may be used wherever required.
7. Teachers should ensure the active participation of students while performing the experiment.
8. Observations should be checked individually and each student should be given a chance to perform the experiment.
9. Teachers should ask the students to complete the questions which are given at the end of the experiment accordingly.
10. Teacher can give more questions to the students at the end of the experiment.
11. Assessment of manuals should be done according to the assessment norms. Proper marks should be distributed according to the performance of the individuals.
12. Teachers should explain the competencies that student should achieve, in detail with their importance to students after completion of their course.
13. Explanation about various equipment with some interesting videos, reagents, chemicals, glassware's should be given to students prior to commencing of the practical.
14. Teachers should observe the students when students are performing practical's in groups, proper contributions of the individual student should be there and record of observation should be noted by all of them.
15. Teachers should also organize a visit to a Government / private healthcare facility to understand and observe the various hospital and clinical pharmacy services provided.
16. Teachers should also ask them to gather information about latest equipments, software used in hospital.
17. Teachers may suggest the students to refer to sources of information such as literature, research papers, books, attending conferences, seminars for the updation of knowledge.
18. According to the professional competencies given by PCI, teachers should develop the professional skills of the students.
19. Teacher should conduct different types of sessions for students such as quiz, group discussions projects on different topics, etc.
20. Teachers should follow Bloom's taxonomy that encourages higher-order thought in their students by building up from lower-level cognitive skills.
21. Teachers should ensure that revised CIAAN– 2017 norms or the latest norms given by MSBTE are followed simultaneously and implemented.
22. Teachers should follow the guidelines given by PCI & MSBTE from time to time.

GUIDELINES OF BLOOMS TAXONOMY LEVELS

1 Knowledge

Define, Identify, Describe, Recognize, Tell, Explain, Recite, Memorize, Illustrate, Quote

2 Understand

Summarize, Interpret, Classify, Compare, Contrast, Infer, Relate, Extract, Paraphrase, Cite

3 Apply

Solve, Change, Relate, Complete, Use, Sketch, Teach, Articulate, Discover, Transfer

4 Analyze

Contrast, Relate, Devise, Distill, Correlate, Illustrate, Conclude, Categorize, Connect, Take apart

5 Evaluate

Criticize, Reframe, Judge, Defend, Appraise, Value, Prioritize, Plan, Grade,

6 Create

Design, Modify, Role-play, Develop, Rewrite, Pivot, Modify, Collaborate, Invent, Write

INSTRUCTIONS TO STUDENTS

Students should follow the instructions given below for better understanding of the subject from a theoretical and practical concept of view.

1. Listen carefully to the lecture given by teacher about importance of subject, graphical structure, skills to be developed, information about equipment, instruments, procedure, method of continuous assessment, tentative plan of work in laboratory and total amount of work to be done in a year.
2. Teacher act as a simulator only, practical significance, and theoretical background must read by the student a day in advance and perform practical by using resources. Thereafter ask the queries to the Teacher.
3. Understand and organize the work in the group and make a record of all observations.
4. Students should actively participate in group activities, role play, discussions, etc. and strive to achieve the knowledge, skills, and attitude.
5. The practical applications of every experiment should be noted by the students.
6. Write the answers of the questions allotted by the teacher during the same practical hours if possible or afterwards, but immediately.
7. Student should submit the manual for assessing regularly on the scheduled date.
8. Student should not hesitate to ask any difficulty faced during conduct of practical / exercise.
9. Student shall refer periodicals /journals / pharmacopoeias, magazines, proceedings of the seminars, refer websites related to the scope of the subjects and update their knowledge and skills.
10. Student should develop the habit of not to depend totally on teachers but to develop self learning techniques.
11. Students should develop different types of competencies to become competent Pharmacists.
12. Student shall visit the hospitals and should make a project report on it as directed by the teacher.

LABORATORY MANUAL OF PHARMACOLOGY
MAPPING OF COURSE OUTCOMES

Sr. No.	Title of the experiment	CO1	CO2	CO3	CO4
	General experimental pharmacology				
1	Introduction to experimental pharmacology	√	√	√	√
2	Study of laboratory animals	√	√	√	√
3	To study the commonly used instruments in experimental pharmacology		√	√	√
4	Study the different routes of administration of drugs in animals. Part-I	√	√		√
5	Study the different routes of administration of drugs in animals. Part-II	√	√	√	√
6	Types of pre-clinical experiments: In-Vivo, In-Vitro, Ex-Vivo, etc.		√		√
7	Techniques of blood collection from animals		√		√
	Effects of various drugs on the Rabbit				
8	Study of local anaesthetics on rabbit eye	√			
9	Study of Mydriatic effect on rabbit eye	√			
10	Study of Miotic effect on rabbit eye	√			
11	Pyrogen testing by rabbit method				√
	Effects of various drugs on the Mice				
12	Effect of analgesics using tail flick apparatus		√		
13	Effect of analgesics using hot plate apparatus		√		
14	Study of analgesic activity by writhing test		√		
15	Screening of anti-convulsant using Electro Convulsiometer		√		
16	Screening of Muscle relaxants using Rota-Rod apparatus		√		
17	Screening of CNS stimulants using Actophotometer		√		
18	Screening of CNS depressants using Actophotometer		√		
19	Study of anxiolytic activity using elevated plus maze method		√		
	Effects of various drugs on the Frog				
20	Study of effect of sympathomimetics (adrenaline) on isolated heart			√	√
21	Study of effect of Parasympathomimetics (acetylcholine) on isolated heart			√	√
22	Study of effect of calcium and potassium ions on isolated heart			√	√
23	Study of effect of cardiotonics on isolated heart			√	√
24	Effect of drugs (acetylcholine) on ciliary motility on frog's buccal cavity/oesophagus			√	
25	Effect of drugs (Atropine) on ciliary motility on frog's buccal cavity/oesophagus			√	

LIST OF EXPERIMENTS AND RECORD OF PROGRESSIVE ASSESSMMENT

Sr. No.	Title of the experiment	Page no.	Date of performance	Date of submission	Assessment Marks	Teacher signature
	General experimental pharmacology					
1	Introduction to experimental pharmacology	1				
2	Study of laboratory animals	5				
3	To study the commonly used instruments in experimental pharmacology	10				
4	Study the different routes of administration of drugs in animals. Part-I	16				
5	Study the different routes of administration of drugs in animals. Part-II	21				
6	Types of pre-clinical experiments: In-Vivo, In-Vitro, Ex-Vivo, etc.	29				
7	Techniques of blood collection from animals	35				
	Effects of various drugs on the Rabbit					
8	Study of local anaesthetics on rabbit eye	42				
9	Study of Mydriatic effect on rabbit eye	46				
10	Study of Miotic effect on rabbit eye	51				
11	Pyrogen testing by rabbit method	56				
	Effects of various drugs on the Mice					
12	Effect of analgesics using tail flick apparatus	61				
13	Effect of analgesics using hot plate apparatus	65				
14	Study of analgesic activity by writhing test	69				
15	Screening of anti-convulsant using Electro Convulsiometer	73				
16	Screening of Muscle relaxants using Rota-Rod apparatus	78				

17	Screening of CNS stimulants using Actophotometer	82				
18	Screening of CNS depressants using Actophotometer	86				
19	Study of anxiolytic activity using elevated plus maze method	90				
	Effects of various drugs on the Frog					
20	Study of effect of sympathomimetics (adrenaline) on isolated heart	94				
21	Study of effect of Parasympathomimetics (acetylcholine) on isolated heart	100				
22	Study of effect of calcium and potassium ions on isolated heart	104				
23	Study of effect of cardiotonics on isolated heart	108				
24	Effect of drugs (acetylcholine) on ciliary motility on frog's buccal cavity/oesophagus	113				
25	Effect of drugs (Atropine) on ciliary motility on frog's buccal cavity/oesophagus	117				

I) PRACTICAL RECORD MARKS*:

Sessional Exam	Experiment No.		Total no. of experiment conducted	Average marks obtained for the experiment conducted. (out of 10)*	Teacher's Signature
	From	To			
First Sessional					
Second Sessional					
Third Sessional					

*Sessional wise marks should be considered for internal assessment of practical sessional examinations (out of 10M)

II) ASSIGNMENT MARKS#:

Sr. No.	Title of Assignment	Marks out of 10#	Assignment Marks (Average of three)	Teacher's Signature
1				
2				
3				

#Marks should be transferred from Appendix -1 A typical format for assessment of an assignment.

Average Sessional Mark out of 10	Assignments Mark out of 10 (Average of three)	Total Marks out 20	Teacher's Signature

Experiment No. 1
Introduction to Experimental Pharmacology

1. Aim:

To get introduced to the experimental Pharmacology

2. Practical Significance:

Experimental pharmacology is one of the cornerstones of the drug discovery process. The medicinal chemist may create the candidate compound, but the pharmacologist is the one who tests it for physiologic activity. A promising compound is investigated by many other scientists-toxicologists, microbiologists, clinicians-but only after the pharmacologist has documented a potential therapeutic effect. The practical in Pharmacology help the students to understand the process of drugs evaluation and identifying the effects produced by it using various experimental animal models. The laboratory helps the pharmacologists to test physiologic effects of the drugs, agents isolated from the plants, extracts of the plants, synthesized compounds from the medicinal chemist, etc. This practical will educate the students about the importance of experimental pharmacology and its scope.

3. Practical Outcomes (PrOs):

After completion of this practical, the students will be able to:

PrO	Practical Outcomes	Mapped CO	BTL
PrO1	Discuss the scope of Experimental Pharmacology.	CO1-4	BTL2
PrO2	Search the internet for getting information of Experimental Pharmacology.	CO1-4	BTL3
PrO3	Create the chart of landmarks in the field of Experimental Pharmacology	CO1-4	BTL6
PrO4	Collaborate and communicate with fellow students.	CO1-4	BTL5

4. Relevant Theoretical Background:

Pharmacology is the science deals with the study of drugs. It broadly covers the information about the history, source, physiochemical properties, mechanism of action, absorption, distribution, metabolism and excretion of drugs.

Drugs are chemical agents used for the purpose of diagnosis, prevention relief or cure of a disease in man or animals. The word drug is derived from the French word ‘drogue’ meaning herb.

Experimental Pharmacology :

Francois Magendie, a French physiologist, was one of the first to use animals in scientific research, performing the first pharmacological experiment on dogs to study the action of a strychnine-containing plant –Nux vomica and demonstrating that the spinal cord was the site of its convulsant action.

Oswald Schmiedeberg (1838-1921), a Baltic German pharmacologist recognized as founder of modern pharmacology. He was the first to introduce the concept of “pharmacodynamics” and “pharmacokinetics” of a drug by studying the action of parasympathomimetic drugs, muscarine and nicotine; digitoxin, hypnotics and analeptics.

Rudolf Buchheim established the first experimental pharmacology laboratory at his home in Germany in 1849. Buchheim transformed medicine from a merely descriptive and empirical study to an experimental science. He stated that the best way to study a drug's effects was to isolate the molecule, investigate its chemical composition, and correlate the drug's chemistry with changes in organ function.

The introduction of the kymograph and methods for maintaining an isolated organ alive later proved to be landmarks in experimental pharmacology. The kymograph was the first apparatus used to record the results of studies, and it was essential in the development of pharmacology as a separate science. Pharmacologists began using new kymographic methods of documenting drug effects in physiological tests, registering changes in physiological function caused by drug delivery as a variable in time.

In an organ/tissue bath containing a physiologic salts solution through which oxygen was bubbled, an isolated organ or tissue remained functioning for several hours. Henrick Magnus (1802–1870) was the first to use this procedure on a tiny intestinal strip, followed by Jean-François Heymans (1904) on the mammalian heart, and Claude Bernard on isolated nerve-muscle preparations. These were the significant advancement in experimental pharmacology methods.

Scope of Experimental pharmacology:

Experimental pharmacology is relatively the youngest branch of basic medical sciences. Electrophysiology, biochemistry, molecular biology, and electronic or digital recording methods and software have all contributed to the enrichment and broadening of experimental pharmacology's frontiers.

The main aims of the experimental pharmacology are to

a. Find out a therapeutic agent suitable for human use
b. Study the toxicity of a drug
c. Study the mechanism of action of drugs

New chemical research is on-going to improve health care and produce more effective, safe, and inexpensive pharmaceuticals. Because experimental pharmacology entails the development of new drugs or the investigation of the effects of existing drugs, it is divided into two stages:

a. Preclinical experimental pharmacology, which entails the identification and optimization of novel chemical lead structures, as well as testing them for biological effects on animals and animal tissues or organs.
b. Clinical pharmacology, involving testing medications on human volunteers and patients to determine their pharmacokinetics, safety, and efficacy.

Animals are not easily available nowadays due to animal welfare regulations and ethics. Animal experiments are expensive, time-consuming and tedious. Thus, with the changing scenario along with the advances in computer technology, alternative methods of teaching pharmacology experiments have been developed. Demonstrations employing computer- simulated learning systems can be used in place of animal tests.

For computer simulation demonstrations, a variety of applications are available. Students can be taught through exercises in the form of graphs and tables derived from numerous animal experiments.

The biological responses are so complicated that accurately simulating genuine animal studies on a computer is rather difficult. Many factors come into play when it comes to controlling an organ or a system.

As a result, the findings obtained using these generated models could be inaccurate.

5. Resources Required:

Watch the video of "Introduction to Experimental Pharmacology" using YouTube video (https://youtu.be/O6DYNOF5GeI) or any other video/software/MSBTE CD available.

6. Resources Used:

_____

_____

7. Observations:

Complete the following table by adding the best discoveries of the scientist in the corresponding column.

Sr. No.	Name of the scientist	Discoveries
1.	Henrick Magnus (1802–1870)	
2.	Claude Bernard (1813-1878)	
3.	Rudolf Buchheim (1820-1879)	
4.	John Jacob Abel (1857-1938)	
5.	Ram Nath Chopra (1882-1973)	

8. Conclusion:

_____

_____

9. References:

a) Kulkarni SK. Hand book of experimental pharmacology. Vallabh Prakashan, Delhi

b) Bronswijk and Cohen. The first recordings of pharmacological effects. British Journal of Clinical Pharmacology. 2008; 66(5):588–593.

10. Practical Related Questions (Teacher can give more questions to the student):

a) Define pharmacology and experimental pharmacology.

b) Explain the first experiment conducted in animals.

c) Give the objectives of experimental pharmacology.

d) Mention the scope of experimental pharmacology.

e) Explain different aspects of drug covered in pharmacology.

(Space for answers)

11. Assessment Scheme:

Particular	Understanding the basic concept (Intellectual skill)	Activities (Intellectual and motor skill)	Cleanliness & Handling (Affective domain)	Viva-voce	Total	Signature of teacher
Marks obtained						
Max marks	**2**	**5**	**1**	**2**	**10**	

Experiment No. 2
Study of Laboratory Animals

1. Aim:

To study the laboratory animals: a) Mice; b) Rats; c) Guinea pigs and d) Rabbits

2. Practical Significance:

When there is no other method to do the research, animals are used. There are four main reasons why the animals are used in research. They are as follows:

1) To improve the understanding of biology;

2) To develop the models to study the diseases;

3) To develop and test potential forms of treatment; and

4) To ensure the safety of people, animals and the environment.

In this experiment, the students will learn about the animals and their use in specific experiments to test the various classes of drugs.

3. Practical Outcomes (PrOs):

After completion of this practical, the students will be able to:

PrO	Practical Outcomes	Mapped CO	BTL
PrO1	Explain the importance of animals in the testing of drugs.	CO1-4	BTL2
PrO2	Discuss the choice of laboratory animals for the given experiment.	CO1-4	BTL2
PrO3	Choose the suitable animals for given experiment.	CO1-4	BTL6
PrO4	Practice ethical behaviour and cleanliness in the laboratory	CO1-4	BTL5

4. Relevant Theoretical Background:

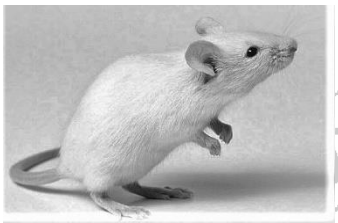
The animals are utilized for a variety of reasons, including scientific study. Animals can also be utilized to learn more about living things and diseases that affect humans and other animals. It is possible to discover information that cannot be learnt by any other way but by studying animals. When a novel drug or surgical method is discovered, society considers it unethical to test it first in humans due to the risk of harm rather than benefit. Instead, animals are used to test the drug or undergo procedure to ensure that it is both safe and effective.

Some of human diseases can't be modelled in experimental animals. Moreover, animals can be fed diets that are identical and constantly monitored. Members of several animal species are genetically identical, similar to inbred mice, allowing researchers to evaluate different techniques on identical animals. Some animals, such as rabbits for atherosclerosis and monkeys for polio, have biochemical similarities to humans that make them particularly drugs models for specific diseases. Monkeys were used to produce the polio vaccine, and it is currently being studied for safety. Animals are particularly crucial in the fast-growing sector of biotechnology, where they are employed to develop, test, and manufacture new biologicals like monoclonal antibodies.

The common laboratory animals are discussed here:

Albino mouse (*Mus musculus*):

The smallest laboratory animals utilized are white mice. Mice are also simple to breed and keep. Because they are little (25-30 g), they are simple to breed and care for. Most medicines used in experimental pharmacology are sensitive to them. Mice are commonly employed in toxicity studies, insulin bioassays, analgesic testing, CNS active drug testing, and chemotherapeutic agent testing. In neuro pharmacology, mouse brain and primary cell culture of mouse spinal cord neurons have lately been employed to examine neurotransmitter receptor activities. Laca and balb/C are the other mouse strains tested.



Albino rat (*Rattus norvegicus*):

White rat (200-250g) is the commonest laboratory animal used in experimental pharmacology. Rats are easy to breed and maintain. Resemble man in several organ function and nutrition and sensitive to most of the drugs; make them very useful experimental animals. However they do not have vomiting centre. The various rat tissue used are colon, stomach, uterus, caecum and vas deference. Besides these organs rat brain tissue is extensively employed in radio receptor ligand studies. The other strains of rats are Sprague-Dawley, Wistar and Porton.

Guinea pig (*Cavia porcellus*):

Guinea pigs (400-600g) are very docile and easy to raise and maintain. They are commonly used experimental animals for the anaphylactic and immunological studies because they are highly sensitive to histamine and penicillin. They are used in experimental asthma to study bronchodilators. It is animal of choice for the bioassay of digitalis. They are also used to study local anaesthetics and as a model in amoebiasis and cholera as they are sensitive to these microorganisms.

Rabbit (*Oryctolagus cuniculus*):

Domestic rabbits (2-3 kg) are generally used for pyrogen testing. Some of the tissues or organs from rabbits used are heart, aorta, duodenum and ileum. One peculiar thing about rabbits is that they are resistant to the actions of atropine as they contain atropine-esterase enzyme, the presence of which is genetically determined.

Other animals :

Other animals like cats, dogs and monkeys are also used for pharmacological investigations of drugs. Cats and dogs were commonly used to study blood pressure experiments. But their use has been now restricted. However, beagle dogs are the only strain approved by regulatory authorities for preclinical testing of new drugs.

Alternatives to animal experimentation :

There is growing concern on the use of animals in biomedical research; hence several countries have passed legislation prohibiting or restricting animal experimentation. The alternatives to experimental animals are widely available and they include experiments with natural animal tissue and body fluids, as well as human usage of microbes, primary cell culture and cell lines, modelling, and computer simulation and software.

Physiological parameters of the laboratory animals:

Parameters	Mouse	Rat	Guinea pig	Rabbit
Body weight	25-30 g	200-300 g	500-800 g	2-3 kg
Life span	1-2 years	2-3 years	2 years	4-5 years
Urine excretion	1-3 ml/d	10-15 ml/d	100-150 ml/d	200-300 m/d
Oestrus cycle	4-5 days	4-5 days	15-19 days	Spontaneous ovulation
Gestation period	19 days	21 days	67 days	32 days
Breeding age	40-56 days	60-80 days	1 year	90-120 days
Body temperature	37.5 ˚C	37.5 ˚C	38.3 ˚C	38.3˚C
Blood volume	70-80 ml/kg	50-65 ml/kg	65-90 ml/kg	45-70 ml/kg

5. Resources required :

a. Read the freely available article by S. Sivakrishnan, S. Vigil Anbiah, (2021), "Animals Used in Experimental Pharmacology", Pharmacophore, 11(1), 1-7.

b. Watch the video of "Study of laboratory animals" using the YouTube video or any other Video / software / MSBTE CD available.

6. Resources used:

_____

_____

7. Observations:

Complete the following table by adding the list of some experiments performed using the given animals.

Sr. No.	Animal	Experiments performed
1.	Albino mouse	a. b. c.
2.	Albino rat	a. b. c.
3.	Guinea pig	a. b. c.
4.	Rabbit	a. b. c.
5.	Monkeys	a. b. c.

8. Conclusion:

_____

_____

9. References:

a) Kulkarni SK. Hand book of experimental pharmacology. Vallabh Prakashan, Delhi
b) Guide for the care and use of laboratory animals, Eighth Edition, The national academic press, Washington, D.C.

10. Practical Related Questions (Teacher can give more questions to the student):

a) List the other animals than discussed here used for the testing of drugs.
b) Give reason, why rabbits are used for pyrogen testing.
c) Mention the various experiments carried out on mice.
d) Write experimental uses of the guinea pigs.
e) Why genetically modified animals are used for experimentation in pharmacology?

(Space for answers)

11. Assessment Scheme:

Particular	Understanding the basic concept (Intellectual skill)	Activities (Intellectual and motor skill)	Cleanliness & Handling (Affective domain)	Viva-voce	Total	Signature of teacher
Marks obtained						
Max marks	**2**	**5**	**1**	**2**	**10**	

Experiment No. 3
Commonly Used Instrument in Experimental Pharmacology

1. Aim:

To study the commonly used instruments in experimental pharmacology

2. Practical Significance:

There is tremendous growth in the electronic devices and recording systems used in the pharmacology practical. Traditionally the smoked paper (kymograph) was used for recording, but now days, recording is done using ink pen and white paper.

The various experimental models have been developed to study the effects of drugs in animals according to their behaviour. Some modifications are done to record the readings automatically that reduces the manual errors and biasness of the experimenter. After going through this practical, the students will learn the basic instruments and equipment that are used in pharmacology laboratory.

3. Practical Outcomes (PrOs):

After completion of this practical, the students will be able to:

PrO	Practical Outcomes	Mapped CO	BTL
PrO1	Identify the important instruments and equipment used in pharmacology laboratory	CO1-4	BTL2
PrO2	Describe the Sherrington rotating drum machine, and organ bath assembly.	CO1-4	BTL2
PrO3	Select the suitable instruments and equipment for given experiment	CO1-4	BTL3
PrO4	Discuss various methods of anaesthesia used in laboratory animals.	CO1-4	BTL2

4. Relevant Theoretical Background:

Instruments used to record on the isolated tissues

1. Organ bath assembly

The student organ bath is the basic instrument used to study the effects of the drugs on the tissues.

The student organ bath was developed by Rudolph Magnus in 1904. The organ bath assembly is a traditional experimental set-up that is commonly used to investigate the physiology and pharmacology of in vitro tissue preparations. Perfused tissues can be maintained for several hours in a temperature-controlled organ bath. It has following main parts:

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

Outlet Pipe: Outlet drainage is attached at the base of the organ bath with pinching stop cock on it. When opened, it drains out the physiological solution from the organ bath.

Inlet Pipe: An inlet pipe originates from the reservoir and attached with coiling pipe around the organ bath.

It is used to add the physiological solution to organ bath when required.

Aeration tube: Oxygen or air delivery tube that also serves as the tissue holder.

Reservoir: It is usually a glass bottle of 5 liters capacity and is kept 2 to 3 feet high above the level of organ bath. It contains the physiological solution.

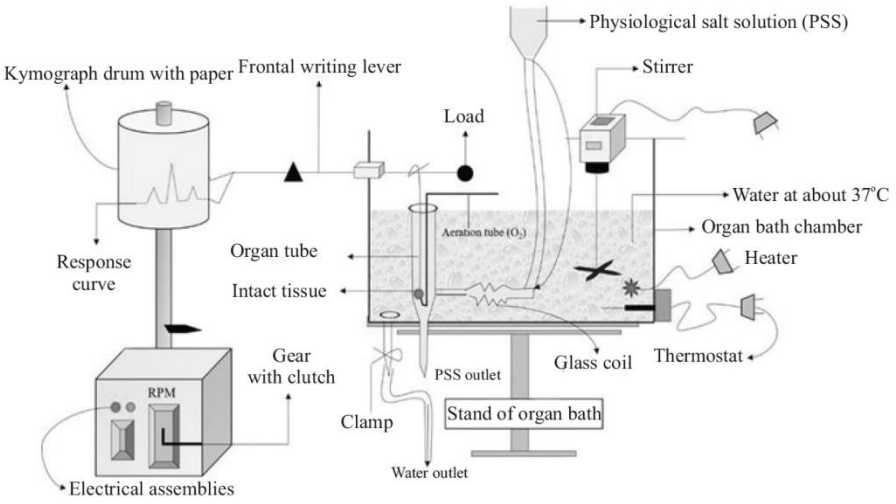


Figure 3.1 Organ Bath Assembly with Sherrington Rotating Drum Machine

2. Sherrington Rotating Drum and Kymograph

This is an electrically driven recording drum machine suitable for use by students for laboratory experiments. It has following parts:

Drive: Its drive mechanism consists of constant speed heavy duty electric motor and an accurate gear box contained in the base together with robust plate clutch. Engagement of various gear ratios are affected by sliding the lever into the slot marked with surface speed or by a knob.

Recording Drum: The recording drum or cylinder has a 6' diameter and stainless-steel spindle. It has been standardized to make the same universal and interchangeable.

Speed Gears: It has 8 variable speeds. The speed is measured in mm per second. Eight variable speeds are 0.12, 0.25, 1.25, 2.5, 12.5, 25, 320 and 640 mm per second. A hand gear is provided to change the speed of the kymograph.

Kymograph paper: It is used to record the recordings from the student's organ bath assembly.

Writing levers are used to trace the recording from muscle contractions.

3. Recording levers

These are used to record the movements (contractions or relaxations) of the isolated tissue preparation. The recording is done on the kymograph or with the help of ink pen on white paper attached to the Sherrington's rotating drum.

Levers are meant for recording and magnifying the responses of isolated tissues to drugs. The levers are attached to isolated tissues and are used to record various types of contractions in them. Types of levers are:

1. Frontal writing lever: This is used for recording the isotonic contractions of the isolated tissues. In this lever, the writing end (stylus) can freely rotate around its axle. This minimizes friction between the stylus and the kymograph. With frontal writing lever, the contractions of the isolated tissues are recorded as straight lines.

2. Simple / Side-way writing lever: This is used for recording isotonic contractions of the isolated tissues. The responses recorded by simple lever are curvilinear. Uncontrolled friction between the writing end (stylus) and the kymograph is a major disadvantage of simple writing lever.

3. Starling's heart lever and Brodie's Universal lever: This is used for recording isometric contractions of the isolated tissues. In this, the horizontal arm of the lever is suspended to a rigid

point with a spring. This type of lever is used for recording rapid and multiple contractions in the isolated tissues.

4. **Gimbal lever:** The friction between the writing end and the kymograph is minimum in the gimbal lever because the pressure of stylus on the kymograph depends on gravity.

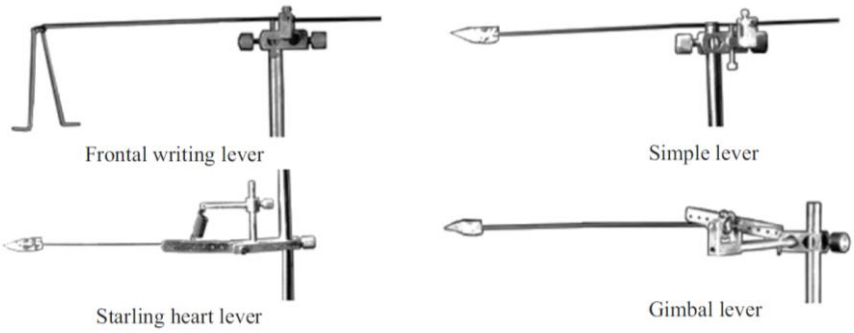
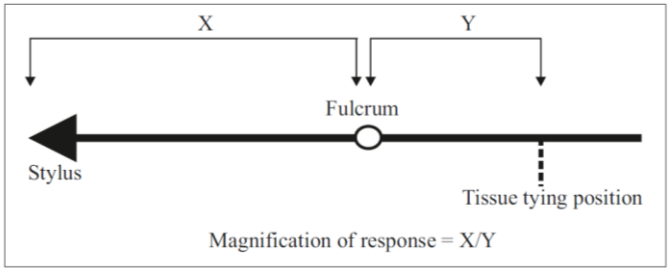


Figure 3.2 Different types of Recording Levers

Magnification of response: The lever has to be adjusted so that contraction recorded on the kymograph is magnified at least 5 times that of the actual contraction of isolated tissue. The magnification of response depends on the ratio of distance between the stylus and fulcrum (X) to the distance between the fulcrum and the tissue tying position (Y).



Different Instruments/Apparatus Used for study of Animal Behavioural Models

1. Elevated plus maze apparatus: This apparatus is used to test the antianxiety drugs.

2. Open field test apparatus: This apparatus is used to test the antianxiety drugs and also test their effects on locomotion.

3. Actophotometer: This is used to record the ambulation of animal and used to test sedatives and hypnotics.

4. Tail flick apparatus: It is used to test the analgesics.

5. Hot plate method apparatus: It is also used to test the analgesics.

6. Rota-rod apparatus: It is used to test the muscle coordination and therefore used for testing the muscle relaxant effect of drugs.

7. Electro-convulsiometer: It is used to induce convulsions by administering the shock in animals and hence used for testing anticonvulsant drugs.

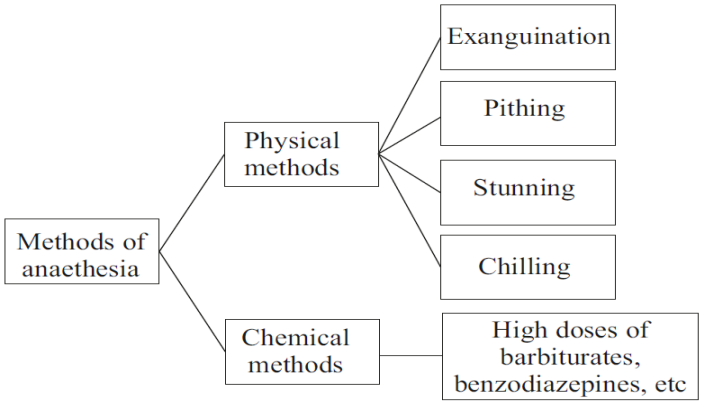
8. Pole climbing apparatus: This apparatus works on the conditioned response by the animals to a specific stimulus. It is used to test the antipsychotic drugs.

Various Methods of Anaesthesia

Anaesthesia: It is the reversible loss of pain sensation achieved by depressing the functioning of reticular activating system.

Euthanasia: It is the process of killing the animals without causing pain. This is also called as mercy killing.

Methods of Anaesthesia



Exsanguination: This is the process of draining blood from the animal or organ. After the animal is unconscious, exsanguination procedure is initiated to ensure death using a pointed, very sharp knife with a rigid blade at least 6 inches (15 cm) in length. Exsanguination procedures are required with the use of penetrating captive bolt. The knife should be fully inserted through the skin just behind the point of the jaw and below the neck bones. From this position the knife is drawn forward severing the jugular vein, carotid artery, and windpipe. Properly performed, blood should flow freely with death occurring within a few minutes.

Chilling: This is one of the methods to decrease the pain sensation in the animals. Animal like frogs are kept at 4˚C for a half hour and then the pithing is performed.

Pithing: This technique is designed to cause death by increasing the destruction of brain tissue. It is performed by inserting a pithing needle through the entry site (occipitoatlantic junction) produced in the skull by the penetrating captive bolt stunner. The pithing needle should be moved up to the spinal cord to destroy both brain stem and spinal cord tissue which ensures death. This procedure is sometimes used in advance of exsanguination procedures to reduce involuntary movement in stunned animals.

Stunning: Stunning is a technique to make animal immobile or unconscious, with or without killing them. This can be done by holding the animal form the back and hit it on a solid platform in way so that stroke should be given in between the head and neck. Immediately the animal is put on platform. The head of animal is hold by one hand tightly and the tail is pulled to destroy the spinal cord connections. This process is a way that dissection thereafter causes no pain to the animal.

5. Requirements:

Organ bath assembly, various types of levers, actophotometer, elevated plus maze apparatus, tail flick apparatus, hot plate method apparatus, rota-rod apparatus, etc.

Watch the video of “Commonly used instruments in experimental pharmacology” using the YouTube video or any other video/software/MSBTE CD available.

6. Requirements used:

_____

_____

7. Observations:

a) Draw a well labelled diagram of the Sherrington rotating drum machine.

b) Draw well labelled diagram of any two-apparatus mentioned in this experiment.

8. Conclusion:

The various laboratory instruments were studied along with their use in experimental pharmacology.

9. References:

a) Kulkarni SK. Practical Pharmacology and Clinical Pharmacy. First Edition, Vallabh Prakashan, Delhi.

b) mycalpharm.com by infokart India Pvt. Ltd. & Xplo Remedies LLP.

10. Practical Related Questions (Teacher can give more questions to the student):

a) Write some others types of the levers used for recording of isolated tissue preparations.

b) Name the apparatus used for the testing anxiety and antianxiety drugs.

c) Which instrument is used to test the effects of sedatives and hypnotics?

d) Name the apparatus used for testing the muscle relaxation property of drugs.

e) Which apparatus is used to test the analgesic effects of centrally acting analgesics?

(Space for answers)

11. Assessment Scheme:

Particular	Understanding the basic concept (Intellectual skill)	Activities (Intellectual and motor skill)	Cleanliness & Handling (Affective domain)	Viva-voce	Total	Signature of teacher
Marks obtained						
Max marks	**2**	**5**	**1**	**2**	**10**	

Experiment No. 4
Different Routes of Drug Administration

1. Aim:

To study the different routes of administration of drugs in animals.(Part I)

2. Practical Significance:

There are different routes of drugs administration in animals. The selection of the route is dependent on the chemical properties of the drug, the physiological effects and nature of the study. After going through this practical students will be familiarize with the various routes of administration, procedure of administration, identify the site for administration, volume of administration, procedure of administration, etc.

3. Practical Outcomes (PrOs):

After completion of this practical, the students will be able to:

PrO	Practical Outcomes	Mapped CO	BTL
PrO1	Explain the procedure of administration of drugs by various routes	CO1-4	BTL2
PrO2	Choose the correct site for the administration of drugs.	CO1-4	BTL3
PrO3	Practice ethical behavior and cleanliness in the laboratory.	CO1-4	BTL5
PrO4	Collaborate and communicate with fellow students	CO1-4	BTL5

4. Relevant Theoretical Background:

The administration of drugs to laboratory animals is often a significant component of experimental design. The chemicals that may be administered are antimicrobials, pharmacological agents anaesthetics, and analgesics; chemical test agents; electrolytes and other fluids; and nutritional support. The dosing methodology is an important consideration when planning an experiment because substances may be given to the same animal or multiple animals in the same study multiple times. Numerous factors should be considered while delivering the drugs to animals. They are absorption, distribution, metabolism and excretion of therapeutic or chemical agents; route, volume, and frequency of administration; duration of treatment; pH, stability, homogeneity, and osmolarity of the substance to be administered; selection of vehicle or solvent for delivering substances that cannot be administered in a solid or particulate state; solution preparation, including considerations for sterility if the substance is being administered parenterally ; and dosing apparatus and animal restraint necessary for specific routes of delivery. In this practical, the students will learn about the delivery of chemical substance to laboratory animals and summarize the practices for various routes of administration to a range of species.

Route of drug administration

A multiple of ways are used to deliver medications to laboratory animals. A critical consideration in deciding the route is whether the medication is being supplied for a local or systemic (either enteral [via the digestive tract] or Parenteral [beyond the digestive tract]) effect. The Parenteral administration methods bypass the first-pass effect of hepatic metabolism and therefore often produce the highest bioavailability of drugs, in contrast to the orally delivered chemicals and treatments, which undergo first pass metabolism. Parenteral methods also avoid some of the variability that comes with enteral absorption. Further more, depending on the goal of the study, regulatory requirements may influence the approach used. For example, nonclinical safety testing,

in which the route of delivery to animals should closely resemble the projected route of administration to humans.

A substance may be given by following routes of administration:

1. Orally (into the mouth) or gastric gavage (delivered directly into the stomach)
2. Intravenous (delivered into a blood vessel)
3. Epicutaneous (delivered onto the skin)
4. Intradermal (delivered into the skin)
5. Subcutaneous (delivered under the skin)
6. Transdermal (delivered across the skin)
7. Intramuscular (delivered into a muscle)
8. Transcorneal or intraocular (instilled onto or into the eye respectively)
9. Intracerebral (into the brain)
10. Epidural (the space surrounding the dura mater)
11. Intrathecal (the space surrounding the distal spinal cord)
12. Intraperitoneal (administered into the peritoneal cavity)
13. Intranasal (sprayed into the nose for absorption across the nasal mucous membranes)
14. Intratracheal (delivered into the lungs by direct tracheal instillation); or administered by a range of lesscommon routes using other body orifices and surgical exposures.

Recommended volumes and sites of administration of substances to laboratory animals

Route	Species	Optimal volume (range)	Site(s)
Gavage	All	5 ml/kg (to 20 ml/kg)	Intragastric
Intravenous	All	Up to 5 ml/kg (bolus) 2 ml/kg hourly (to 4 ml/kg/h) continuous infusion)	Rodents: tail or saphenous vein Rabbits: ear or cephalic vein
Subcutaneous	All	Maximum of 5 ml/kg per site	Intrascapular, neck, shoulder, flank
Intramuscular	All	Maximum of 0.05 ml/kg per site (rodents, rabbits)	Triceps, quadriceps, dorsal lumbar, semimembranosus, semitendinosus muscles
Intraperitoneal	All	Maximum of 10 ml/kg	Peritoneal cavity
Intranasal	Rodents Rabbits	Minimum of 35µl per animal (50 µl) 200 to 500 µl per animal	Nose

5. Precautions:

- Some drugs may have adverse side effects or cause discomfort if injected via a non-recommended route, hence use the manufacturer's recommended route of injection.
- The volume to be injected should be the lowest volume possible and not exceed the current recommended guidelines (see the table).
- All substances for injection should be sterile since contamination can cause infection and irritation at the site of injection and cause clinical illness in the animals.
- Warm substances to room or body temperature since injection of cold substances can cause discomfort and drop in body temperature (if this does not damage drug).

6. Requirements:

- Feeding tubes and syringes (appropriately sized for injection volume); Needles (appropriately sized for animal); 70% isopropyl alcohol (to disinfect top of multi-dose vial); Gauze; Heat source to warm substances to be injected (do not overheat beyond 37˚C); Heating pad, water bath, holding vial in hand to warm. Optional: towel for wrapping rats if only 1 person is giving the injection.
- Watch the video of "Different Routes of Drug Administration in Animals" using the YouTube video or any other video/software/MSBTE CD available.

7. Resources used:

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8. Procedure:

Intragastric administration

Mouse: 18-20 gauge feeding tubes about 1.5 inches in length with a rounded tip (see the figure). If gavage is performed on young mice a smaller tube is used.

Rat: 16-18 gauge feeding tube about 2-3 inches in length.

For large mice and small rats (30+ grams) an 18-gauge ball tipped gavage needle can be used.

a. Weigh the animal and determine the appropriate dosing volume.

b. Check the length of the gavage tube by measuring from the tip of the animal's head to the last rib. Mark the tube at the nose and do not pass the tube into the animal past that point to avoid perforation of the stomach.

c. To restrain the mouse: scruff the mouse, grasping the skin over the shoulders with the thumb and middle fingers. Grasp the skin over the shoulders so that the fore legs are extended out to the side, keeping the front feet from pushing the gavage tube away. To restrain the rat: hold the rat near the thoracic region and support the lower body.

d. Hold the animal's head in place by gently extending the head back - this extension of the head creates a straight line through the neck and esophagus.

e. Place the gavage tube in the diastema of the mouth. The tube is then gently advanced along the upper palate until the esophagus is reached. The tube should pass easily into the esophagus. The animal may swallow as the tube is passed. Pass the tube smoothly in one motion. Note: If there is any resistance, do not force the tube. Pull the tube out and try again.

f. Once proper placement is verified, the material can be administered by a syringe attached to the end of the tube. Do not rotate the tube because the tip may rupture the esophagus. After dosing, remove the tube gently following the same angle as insertion.

g. Return the animal to the cage and monitor for 5-10 minutes, looking for signs of labored breathing or distress. Monitor animals again between 12-24 hours after dosing.

Figure 4.1 Gavage needle

Figure 4.2 Intragastric administration

9. Observations:

a. Calculate the dose in ml for intragastric administration of a drug from the stock solution of 50 mg/ml to a rat of 260 g. The dose of drug is 100 mg/kg for rat.

Solution

Dose of drug = 100 mg/kg

= 100 mg/ 1000 g

Dose for rat weighing 260 g = 100 × 260 /1000

= 26000/1000 mg

= _____ mg

Drug stock solution = 50 mg/ml

Dose in ml : 50 mg in 1 ml

Dose in _____mg/_____ ml (by cross multiplication)

= 1 × _____ /50

= _____ ml

10. Results:

- _____ ml should be used for intragastric administration of a drug from the stock solution of 50 mg/ml to a rat of 260 g, when the dose of drug is 100 mg/kg.

11. Conclusion:

Different routes of administration of drugs in animals were studied.

12. References:

a) A Good Practice Guide to the Administration of Substances and Removal of Blood, Including Routes and Volumes; Diehl, K et al. 2001

b) Administration of Substances to Laboratory Animals: Routes of Administration and Factors to Consider; Turner, Pekow, Vasbinder, Brabb, 2011

c) Canadian Council on Animal Care (CCAC) guidelines: mice https://www.ccac.ca/Documents/Standards/Guidelines/CCAC_Guidelines_Mice.pdf

d) UBC Guidelines and Standard Operating Procedure

e) mycalpharm.com by infokart India Pvt. Ltd. &XploRemedies LLP.

13. Practical Related Questions (Teacher can give more questions to the student):

a) Write the complications of intragastric administration in animals.

b) If the drug is degraded by the gastric juice enzymes, suggest the route of administration and add note on why?

c) Mention the precautions to be taken while injecting the drug through intragastric route.

d) Give reason, why it is necessary to warm the substance before injection.

e) Mention the merits and demerits of oral route of drug administration.

(Space for answers)

14. Assessment Scheme:

Particular	Understanding the basic concept (Intellectual skill)	Activities (Intellectual and motor skill)	Cleanliness & Handling (Affective domain)	Viva-voce	Total	Signature of teacher
Marks obtained						
Max marks	**2**	**5**	**1**	**2**	**10**	

Experiment No. 5

Different Routes of Drug Administration

1. Aim:

To study the different routes of administration of drugs in animals.(Part II)

2. Practical Significance:

There are different routes of drugs administration in animals. The selection of the route is dependent on the chemical properties of the drug, the physiological effects and nature of the study. After going through this practical students will be familiarize with the various routes of administration, procedure of administration, identify the site for administration, volume of administration, procedure of administration, etc.

3. Practical Outcomes (PrOs):

After completion of this practical, the students will be able to:

PrO	Practical Outcomes	Mapped CO	BTL
PrO1	Explain the procedure of administration of drugs by various routes	CO1-4	BTL2
PrO2	Choose the correct site for the administration of drugs.	CO1-4	BTL3
PrO3	Practice ethical behaviour and cleanliness in the laboratory.	CO1-4	BTL5
PrO4	Collaborate and communicate with fellow students	CO1-4	BTL5

4. Relevant Theoretical Background:

Route of drug administration

A multiple of ways are used to deliver medications to laboratory animals. A critical consideration in deciding the route is whether the medication is being supplied for a local or systemic (either enteral [via the digestive tract] or parenteral [beyond the digestive tract]) effect. The parenteral administration methods bypass the first-pass effect of hepatic metabolism and therefore often produce the highest bioavailability of drugs, in contrast to the orally delivered chemicals and treatments, which undergo first pass metabolism. Parenteral methods also avoid some of the variability that comes with enteral absorption. Furthermore, depending on the goal of the study, regulatory requirements may influence the approach used. For example, nonclinical safety testing, in which the route of delivery to animals should closely resemble the projected route of administration to humans.

5. Precautions:

- Some drugs may have adverse side effects or cause discomfort if injected via a non-recommended route, hence use the manufacturer's recommended route of injection.
- The volume to be injected should be the lowest volume possible and not exceed the current recommended guidelines (see the table).
- All substances for injection should be sterile since contamination can cause infection and irritation at the site of injection and cause clinical illness in the animals.
- Warm substances to room or body temperature since injection of cold substances can cause discomfort and drop in body temperature (if this does not damage drug).

6. Requirements:

- Feeding tubes and syringes (appropriately sized for injection volume); Needles (appropriately sized for animal); 70% isopropyl alcohol (to disinfect top of multi-dose vial); Gauze; Heat source to warm substances to be injected (do not overheat beyond 37˚C); Heating pad, water bath, holding vial in hand to warm. Optional: towel for wrapping rats if only 1 person is giving the injection.
- Watch the video of "Different Routes of Drug Administration in Animals" using the YouTube video or any other video/software/MSBTE CD available.

7. Resources used:

_____

_____

8. Procedure:

1. Intraperitoneal administration

Mouse: 25-26 gauge needle with the volume <10 ml/kg, i.e.: for a 25 gram mouse, the maximum volume would be 0.25 ml.

Rat: 23-25 gauge needle with the volume <10 ml/kg, i.e.: for a 250 gram rat, the maximum volume would be 2.5 ml.

a. Disinfect top of multi-dose vial with 70% alcohol and gauze.

b. Draw up, into the syringe and needle, the amount of solution to be administered. It is helpful to turn the needle so that the bevel points "up" and the numbers on the syringe barrel can be read.

c. Gently remove animal from the cage and restrain appropriately in the head-down position.

For mice: Tilt the mouse with its head slightly towards the ground so that its head is lower than its hind end. This allows the abdominal viscera to shift cranially and minimize accidental puncture of abdominal organs at site of injection.

Fig. 4.1 Intraperitoneal administration in mice

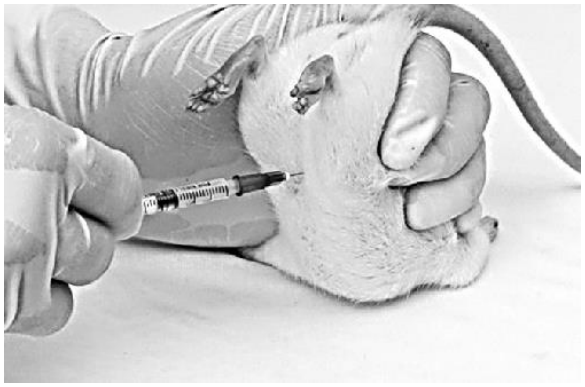


Fig 4.2 Intraperitoneal administration in rats

For rats

The 2 person technique (preferred and recommended)

Use the scissor or 'V' holds method. Hold the head of rat between index and middle fingers (don't press the trachea). Now without squeezing the animal, wrap the remaining fingers gently around the thoracic cavity. Straighten index and middle finger to extend the head and further restrain the rat. With other hand, hold onto the rear feet and tail. Place a finger between legs to reduce stress on the joints. Gently stretch rat along hand/forearm with the head held lower than the body. The second person will perform the injection.

The 1 person technique

Using a small towel, wrap the rat head first as shown below ensuring it cannot get out of the towel, without wrapping too tightly. Gently rotate the rat and towel so the rat is on its back. Place the rat's head/body along the arm and in the crook of the elbow of non-dominant arm. Use arm/elbow to gently restrain the rat against your body. Restrain the feet/tail with non-dominant hand.

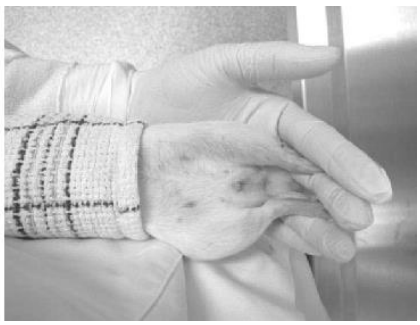


Figure 4.3The 1-person technique

a. Identify anatomical landmarks in order to inject into the appropriate area of the abdomen. Typically, the injection site will be in the animal's lower right quadrant of the abdomen to avoid damage to the urinary bladder, cecum and other abdominal organs.
b. In both mice and rats, insert needle with bevel facing “up” into the lower right quadrant of the abdomen towards the head at a 30-40˚ angle to horizontal. Insert needle to the depth in which the entire bevel is within the abdominal cavity
c. Ensure negative pressure by pulling back the plunger prior to injecting. If there is negative pressure, proceed with the injection (depress the plunger until the solution has been fully administered). The speed of injection depends on the volume and viscosity of the substance. If injecting aqueous solutions, the injection can be completed in 1-2 seconds. Larger volumes or viscous substances may be injected for longer time.

   Do not allow the needle to move around inside the abdomen.

   Aspiration of green material while ensuring negative pressure indicates that the bowel has been punctured, while aspiration of yellow liquid indicates the bladder has been punctured. The presence of blood indicates an abdominal blood vessel has been punctured. If blood, urine or gastrointestinal contents are drawn back into the hub of the needle, remove the needle from the animal. The syringe/syringe contents and needle must be discarded. The angle at which the needle is subsequently inserted should be redirected and the animal is monitored closely for any signs of pain, illness, etc.
d. Pull the needle straight out. Use a new needle and syringe for each animal.
e. Place the animal back into its cage and observe for any complications.

2. Intravenous administration

Mouse: 25-27 gauge needle with the volume 5-10 ml/kg, i.e.: for a 25 gram mouse, the maximum volume would be 0.125-0.25 ml.

Rat: 25 gauge needle with the volume 5-10 ml/kg, i.e.: for a 200 gram rat, the maximum volume would be1-2 ml.

a. Place the mouse or rat in a cage on the heating pad, turned on 'low', for 5-10 minutes or soak the tail in warm water to cause vasodilation (enlargement) of the vein. Ensure the animal does not overheat.

b. Place the animal in the restraining device.

c. Clean the tail with warm water and swab it with alcohol dampened gauze to increase the visibility of the vein.

d. Locate one of the two lateral tail veins in the middle third of the tail.

e. Restrain the tail while occluding the vein with non-dominant hand. With the bevel of the needle facing upward and the needle almost parallel to the vein, slide the needle into the tail vein. Confirm the location by gently applying negative pressure to the plunger; if the needle is in the vein, it is confirmed by observing a flash of blood in the hub of the needle. If do not see a flash of blood in the hub of the needle pull needle backs lightly without removing it from the tail, while keeping negative pressure in the syringe and redirect the needle until a flash of blood is observed.

f. Release the vein occlusion proximal (closer to the animal) to injection site. Slowly press the plunger to inject drug into the vein. If the needle is in the vein, there will be no resistance while injecting and the vein itself will blanch. If the needle is not in the vein, the fluid will cause blanching around the vein or a subcutaneous bleb.

g. Slowly remove the needle from the vein and apply slight pressure to the puncture site with a dry piece of gauze until the bleeding has stopped.

h. Remove the animal from its restrainer and place it in the cage.

i. Monitor the animal for 5- 10 minutes to ensure haemostasis (bleeding has stopped).

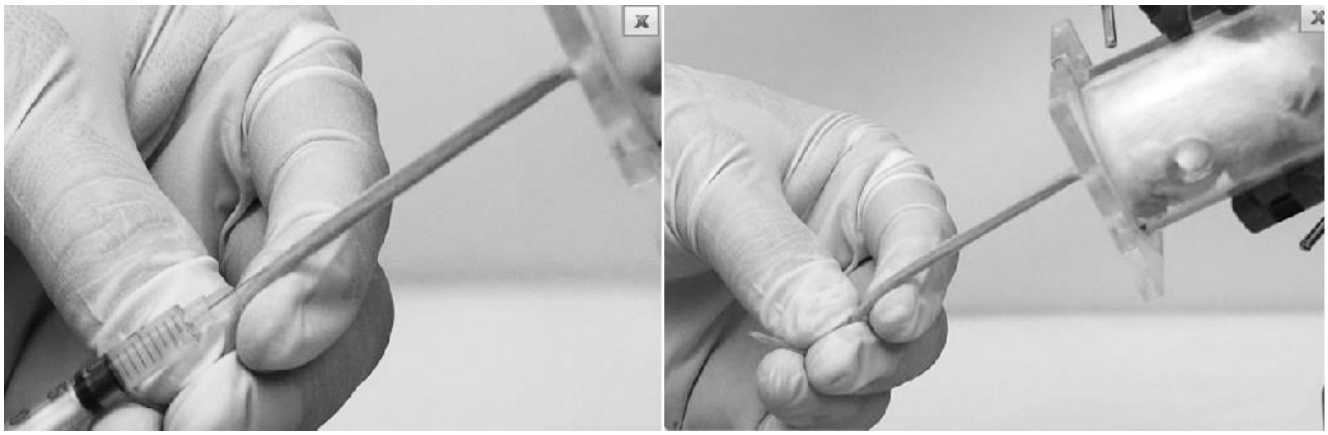


Figure 4.4 Intravenous administration mice

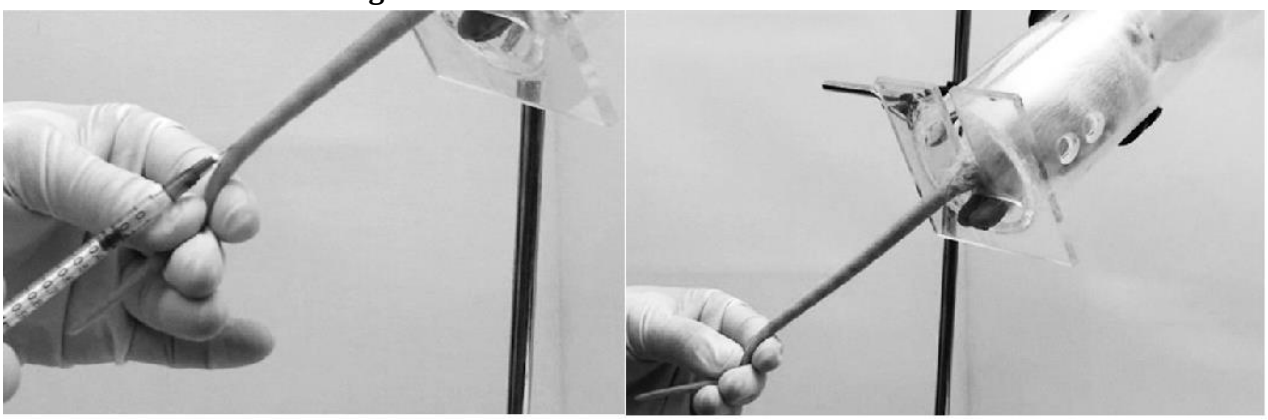


Figure 4.5 Intravenous administration rat

3. Subcutaneous administration

Mouse: 25-27 gauge needle with the volume 5 ml/kg per site, i.e.: for a 25 gram mouse, the maximum volume would be 0.125 ml.

Rat: 23-25 gauge needle with the volume 5 ml/kg per site, i.e.: for a 200 gram rat, the maximum volume would be 1 ml.

a. Weigh the animal and calculate the volume to be administered.

b.Warm up the container of drug to be administered or after filling it in the syringe hold the syringe in between palm to warm.

c.Restrain the animal in an appropriate manner to allow skin access for injection. Use a cloth or small towel to cover the face of rat.

For mouse: "Scruff" the mouse (gather the skin) with non-dominant hand, lifting up the skin to form a "skin tent" and allow access for needle.

For rat: Towel is used to cover the rat's face so it cannot see and become calm. The weave of the towel should allow the rat to breathe once wrapped. Wrap figures around the back of body and tuck it with non-dominant hand so it cannot move (forward or backward) while the injection is being given. Use gentle pressure to keep rat in place and ensure it can breathe easily.

d.Insert the needle into the base of the tented skin with dominant hand. Insert the full length of the needle, parallel to the body, with the bevel facing up.

e.Ensure negative pressure by pulling back the plunger and if nothing is aspirated then do not allow the needle to move further in or out of the skin. If blood is drawn back into the hub of the needle, maybe while injecting needle capillary is get hit. Withdraw and re attempt with a new needle, new syringe and fresh solution. If air is drawn back into the syringe, maybe the skin is exited. Pull the needle out; expel the air, and then re attempt with the same needle. It is recommended to change the needle after 2 attempts and maximum of 3attempts are permitted.

f. If nothing is aspirated then inject the solution slowly.

g. Discard syringe and needle.

h. Return the animal to its cage and observe for any complications

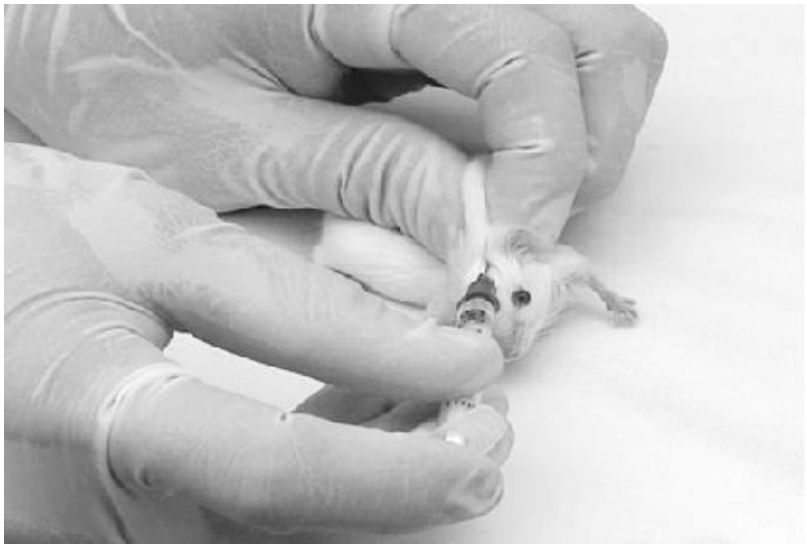


Figure 4.6 Subcutaneous administration

5. Intramuscular administration

Mouse: 25-27 gauge needle with the volume 0.05 ml/kg per site, i.e.: for a 25 gram mouse, the maximum volume would be 0.00125 ml.

Rat: 23-25 gauge needle with the volume 0.05 ml/kg per site, i.e.: for a 200 gram rat, the maximum volume would be 0.01 ml.

a. Restrain the animal by grasping the skin along its back with non-dominant hand.
b. Clean the injection site with alcohol.
c. Insert needle into thigh muscles, and directed away from the femur avoiding the sciatic nerve.
d. Pull back the plunger to aspirate the syringe. Any blood indicates improper needle placement, and needle must be repositioned.
e. Administer substance in a steady, fluid motion. Take care not to administer fluid too rapidly.

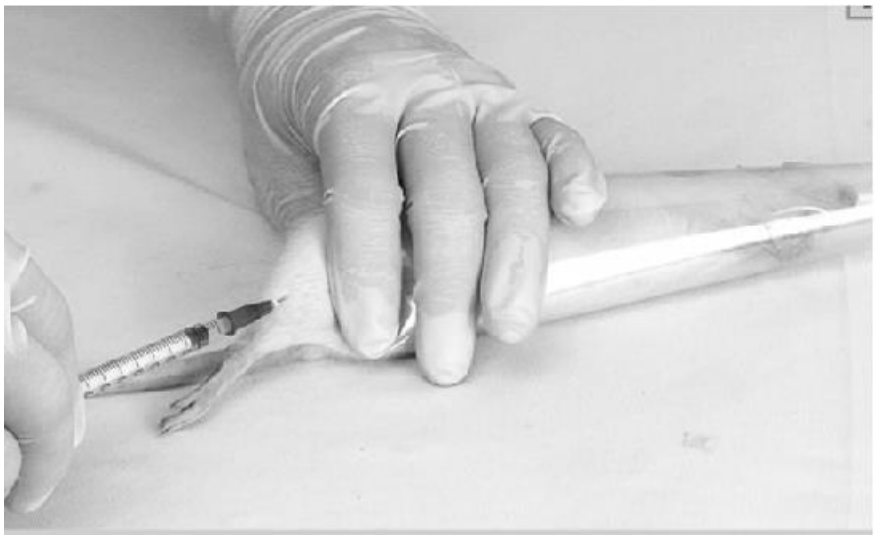


Figure 4.7. Intramuscular administration

9. Observations:

a. Calculate the volume in ml to administer for a 24 g of mouse for the intravenous administration.

Solution

Maximum volume administered to a mouse by IV route is 5 ml

Means Dose = 5 ml/kg = 5 ml/ 1000 g

Dose for mouse weighing 24 g = 5 × 24 /1000

= _____/1000 mg

= _____ ml

b. Calculate the volume in ml to administer for a 20 g of mouse for the subcutaneous administration.

Solution

Maximum volume administered to a mouse by SC route is 5 ml

Means Dose = 5 ml/kg = 5 ml/ 1000 g

Dose for mouse weighing 20 g = 5 × 20 /1000

= _____/1000 mg

= _____ ml

10. Results:

a. _____ ml should be used for the intravenous administration in 24 g of mouse.

b. _____ ml should be used for the subcutaneous administration in 20 g of mouse.

11. Conclusion:

Different routes of administration of drugs in animals were studied.

12. References:

a) A Good Practice Guide to the Administration of Substances and Removal of Blood, Including Routes and Volumes; Diehl, K et al. 2001

b) Administration of Substances to Laboratory Animals: Routes of Administration and Factors to Consider; Turner, Pekow, Vasbinder, Brabb, 2011

c) Canadian Council on Animal Care (CCAC) guidelines: mice
https://www.ccac.ca/Documents/Standards/Guidelines/CCAC_Guidelines_Mice.pdf

d) UBC Guidelines and Standard Operating Procedure

e) mycalpharm.com by infokart India Pvt. Ltd. & XploRemedies LLP.

13. Practical Related Questions (Teacher can give more questions to the student):

a) Write the complications of intravenous administration in animals.

b) If the drug is metabolized by the first pass effect, suggest the route of administration and add note on why?

c) Mention the merits and demerits of IV route of drug administration.

d) Give reason, why it is necessary to warm the substance before injection.

(Space for answers)

14. Assessment Scheme:

Particular	Understanding the basic concept (Intellectual skill)	Activities (Intellectual and motor skill)	Cleanliness & Handling (Affective domain)	Viva-voce	Total	Signature of teacher
Marks obtained						
Max marks	**2**	**5**	**1**	**2**	**10**	

Experiment No. 6
Study of Pre-clinical Experiment

1. Aim:

To study the various types of pre-clinical experiments.

2. Practical Significance:

Preclinical investigators use different methods including experimental animals, tissues, and cell cultures as well as computational simulation studies for finding the ways to treat human diseases and disorders. These studies are *in vitro*, *ex vivo* and *in vivo*. All the procedures have their own advantages and disadvantages. For instance although animal models provide some drawbacks like difference in kinetic parameters or extrapolation of results to human, they are more reliable than *in vitro* tests. The disadvantage of the *in vitro* procedures is that they are mostly performed on cancerous cell lines that have a substantially abnormal function. Furthermore, although *in vitro* models are fruitfully used, finding an end point, finding the effect of chemical, and extrapolation of the effects to the human are some real weaknesses. The present experiment has discussed the pros and cons of these methods, especially in the fields of pharmacology and toxicology.

3. Practical Outcomes (PrOs):

After completion of this practical, the students will be able to:

PrO	Practical Outcomes	Mapped CO	BTL
PrO1	Identify the various preclinical investigation methods.	CO1-4	BTL2
PrO2	Explain the advantages and disadvantages of preclinical investigation method	CO1-4	BTL2
PrO3	Select the suitable preclinical investigation method for the practical.	CO1-4	BTL5
PrO4	Practice ethical behaviour and cleanliness in the laboratory	CO1-4	BTL5

4. Relevant Theoretical Background:

Preclinical investigation methods, also known as non-clinical trials are the laboratory tests of a new drug or of a known substance, device or medical treatment on animal subjects. The main purpose of preclinical studies is to see whether the drug or the treatment is effective and safe to test on humans and for educational purposes. Thus, the main goal of a preclinical research is to collect sufficient data and establish the safety profile of the drug or the treatment under investigation. To fulfil this objective, various types of studies are carried out in a preclinical trial.

These studies can either be *in vitro* ("within the glass"), referring to studies using cell cultures studied outside of the body, or *in vivo* ("within the living"), referring to studies that take place in the body, or *ex vivo* ("out of the living"), referring to studies that take place on functional intact whole organ outside of animal body maintaining physiological conditions.

A) In vitro

In *in vitro* studies, cell lines are derived from either humans or animals and are introduced to the new or old drug or chemical under development within a Petri dish or test tube for investigational or educational purpose.

Common examples of *in vitro* experiments include experiments that uses

a) Cells derived from multi cellular organisms (cell culture or tissue culture),
b) Subcellular components (e.g. mitochondria or ribosomes),
c) Cellular or subcellular extracts (e.g. wheat germ or reticulocyte extracts), or

d) Purified molecules in the test tube (often proteins, DNA, or RNA, either individually or in combination).

Benefits of in vitro studies

- They do not cause harm to the animal or person that the cell cultures have been derived from.
- They are free of the drawbacks of animal testing.
- They are relatively cheap in set up and running the experiments.
- They are also reliable and efficient, and produce robust results.

Drawbacks of in vitro studies

- They are limited as they cannot model how a drug or chemical may interact with all the molecules and cell types that exist within a complex organ.
- They can only be tested in isolation, whereas the human body is a dynamic environment where numerous pathways and cells are in constant communication.
- It is difficult for in vitro studies to predict the complexities of potential interactions.

In vitro investigations, despite this limitation, constitute an important aspect of preclinical research. Preclinical in vitro cancer models have long been known to be unsuccessful at accurately predicting patient outcomes. While in vitro testing in cell-based models has always been an important aspect of cancer research, these preclinical studies have not always produced data that accurately reflects the behaviour of malignancies in response to potential treatments.

B) In vivo

In *in vivo* studies, testing of the substances is carried out using whole animal. These substances maybe new to identify their beneficial effects or known substances testing for the educational purposes.

Animal models like rat, mouse, rabbit, pig, and hamster are used to determine the effects of the testing substance in these animals. After successful results in initial stages, higher animals like cats, dogs, and monkeys are used for preclinical trials. The common examples of the in vivo experiments in the pharmacology and toxicology are determination of analgesic or pain relieving activity in the new drug using analgesiometer; determination of centrally acting agents in various animal models like elevated plus maze, actophotometer, pole climbing apparatus, forced swim test, etc. In these experiments the natural behaviour of animal is used for knowing the effects of drugs. Moreover, the diseased conditions are produced in animals like streptozotocin induced diabetes; strychnine induced convulsions; apomorphine induced psychosis, etc., and then used to test the effects of the new substances. These studies have various beneficial effects than the in vitro experiments.

Benefits of in vivo studies

- They are able to demonstrate the impact of a substance on the body as a whole, rather than how it impacts isolated cells.
- Better visualizations of the potential interactions with biomolecules.
- Better predictions of safety, toxicity, and efficacy.
- Helps scientists predict the impact of substance on human disease.

Drawbacks of in vivo studies

- In vivo studies face significant ethical concerns, particularly for preclinical studies where just animal models are permitted.

C) Ex vivo

In *ex vivo* studies, experiments or testing is carried out, outside of the animals body but maintaining the physiological conditions artificially. There are minimum alterations in the natural physiological conditions. *Ex vivo* conditions allow experimentation under more controlled conditions than is possible in *in vivo* experiments, at the expense of altering the natural physiological environment. The common examples of the *ex vivo* experiments are testing of the drugs on isolated frog heart; on rectus abdominus muscle of frog; rat trachea, experiments on chick chorioallotoic membrane; etc.

Benefits of ex vivo studies

- The main advantage of ex vivo models lie in the fact that there are controlled conditions at all times, i.e., minimum alteration and variation in experimental models.
- Tests and measurements those are not possible to be conducted in living animals due to ethical issues can be easily carried out.

Drawbacks of ex vivo studies

- It is not possible to control every natural variable in the artificial physiologic environment.
- There are chances the substances prove beneficial effects in ex vivo experiments may get fail in *in vivo* experiments.

D) In silico

In silico refers to tests or simulations carried out on a computer or by theoretical analysis, as opposed to "in vivo" (in a real organism) or "in vitro" (in a laboratory). This sort of study has applications in many domains, including drug discovery and development, genetics, and bioinformatics.

Benefits of in silico studies

- Capacity to run tests more rapidly
- Reliable, cost-effective, time-saving, and increasing success rates
- Test ideas that would be difficult or impossible to test in vivo or in vitro

Drawbacks of in silico studies

- Outcomes of in silico trials should be confirmed by experimental or observational investigations

5. Resources Required:

Various charts showing the *in-vivo*, *in-vitro*, *ex-vivo* or *in-silico* experiments. Apparatus or equipment mentioned above.

Watch the video of “Types of pre-clinical experiments” using the YouTube video or any other video/software/MSBTE CD available.

6. Resources used:

_____

_____

7. Observations:

a. Differentiate between *in-vivo, in-vitro* experiments

Sr. No.	*In-vivo* experiments	*In-vitro* experiments

b. Write any five examples of in-vivo, in-vitro and ex-vivo experiments carried out in pharmacology.

Sr. No.	*In-vivo*	*In-vitro*	*Ex-vivo*
1.			
2.			
3.			
4.			
5.			

8. Result:

Types of pre-clinical experiments were studied.

9. References:

a) Gargiulo, G., 2018. Next-Generation in vivo Modeling of Human Cancers. Frontiers in Oncology, 8.
b) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6192385/.
c) Højelse, F., 2000. Preclinical Safety Assessment: In vitro - in vivo Testing. Pharmacology & Toxicology,86, pp.6-7. https://pubmed.ncbi.nlm.nih.gov/10905745/.
d) Lorian, V., 1988. Differences between in vitro and in vivo studies. Antimicrobial Agents and Chemotherapy, 32(10), pp.1600-1601. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC175930/.
e) mycalpharm.com by infokart India Pvt. Ltd. &XploRemedies LLP.

10. Practical Related Questions (Teacher can give more questions to the student):

a) Is ex vivo better than *in vitro*, why?
b) Enlist the various benefits of the *ex vivo* experiments.
c) Name at least five *in vitro* experiments.

d) Why one has to use *in vivo* studies?

e) Give the benefits of *in vivo* studies.

(Space for answers)

11. Assessment Scheme:

Particular	Understanding the basic concept (Intellectual skill)	Activities (Intellectual and motor skill)	Cleanliness & Handling (Affective domain)	Viva-voce	Total	Signature of teacher
Marks obtained						
Max marks	**2**	**5**	**1**	**2**	**10**	

Experiment No. 7
Study of Blood Collection Techniques from Animals

1. Aim:

To study the various techniques of blood collection from animals

2. Practical Significance:

Blood collection from laboratory animals is required for a variety of scientific studies, and there are a number of effective ways available. Because stress affects the study's outcome, it's critical that blood samples from experimental animals be collected in the least stressful way possible. The use of animals and the techniques used for blood collection in laboratory animals have been controlled by a number of regulatory agencies and guidelines. The permitted blood collection techniques for laboratory animals such as rats and mouse are discussed in this practical.

3. Practical Outcomes (PrOs):

After completion of this practical, the students will be able to:

PrO	Practical Outcomes	Mapped CO	BTL
PrO1	Select the suitable site for the collection of blood as per need of testing.	CO1-4	BTL5
PrO2	Discuss the various techniques of blood collection from animals.	CO1-4	BTL2
PrO3	Restrain the animal and collect the blood from it.	CO1-4	BTL5
PrO4	Practice ethical behaviour and cleanliness in the laboratory	CO1-4	BTL5

4. Relevant Theoretical Background:

General principles of blood collection in animals.

a. The method for blood collection used should be least painful and stressful.

b. The blood sample may be collected under anaesthesia or without anaesthesia.

c. In general, blood sample is withdrawn from venous or arterial blood vessels.

d. All non-terminal blood collection without replacement of fluids is limited up to 10% of total circulating blood volume in healthy, normal, adult animals on a single occasion and collection may be repeated after 3to 4 weeks.

e. In case of repeated blood samples as required in pharmacokinetic studies, a maximum of 0.6 ml/kg/day or1.0% of an animal's total blood volume can be removed every 24 hours.

Methods for blood collection in animals

The blood samples are collected using the following techniques:

a) **Blood collection not requiring anaesthesia**
   a. Saphenous vein (rat, mice, guinea pig)
   b. Dorsal pedal vein (rat, mice)

b) Blood collection requiring anaesthesia (local/general anaesthesia)
   a. Tail vein (rat, mice)
   b. Tail snip (mice)
   c. Orbital sinus (rat, mice)
   d. Jugular vein (rat, mice)
   e. Temporary cannula (rat, mice)
   f. Blood vessel cannulation (rat, guinea pig)
   g. Tarsal vein (guinea pig)
   h. Marginal ear vein/artery (rabbit)

c) Terminal procedure

a. Cardiac puncture (rat, mice, guinea pig, rabbit)

b. Orbital sinus (rat, mice)

c. Posterior vena cava (rat, mice)

5. Resources:

Animal, anaesthetic agent, IV cannula, absolute alcohol, rodent handling gloves, towel, cotton, surgical blade, animal warming chamber, sample collection tubes, capillary tube, and 23G or 27G or 20G needle.

Watch the video of "Techniques of blood collection from animals" using the YouTube video or any other video/software/MSBTE CD available.

6. Resources used:

_____

_____

7. Precautions:

a) Aseptic precautions are necessary while collecting blood from lateral saphenous vein.

b) Ensure that all chemical, surgical, fluid requirements are available in the working site, before starting any kind of blood sample collection.

c) Not more than two to three attempts should be made to collect any kind of in vitro biological sample (excluding biological secretion).

d) Label the blood collection tube before starting the experiment and collect blood sample in the appropriately labelled collection tube.

8. Procedures:

A. Saphenous vein blood sample collection

a) Shave the back of the hind leg with electric trimmer until saphenous vein is visible. (Hair removal cream can also be used)

b) Restrain the animal manually or using a suitable animal restrainer.

c) Immobilize hind leg and apply slight pressure gently above the knee joint.

d) Puncture the vein using a 20G needle and collect enough blood with a capillary tube or a syringe with a needle.

e) Compress the punctured site to stop the bleeding.

B. Dorsal pedal vein blood sample collection

a) Keep the animal in a restrainer.

b) Hold the hind foot around ankle and locate the medial dorsal pedal vessel on top of the foot.

c) Clean the foot with cotton dipped in absolute alcohol and puncture the dorsal pedal vein with 23G/27G needle.

d) Collect the drops of blood that would appear on the skin surface in a capillary tube and apply a little pressure to stop the bleeding.

C. Tail vein blood sample collection (Recommended for collecting large volume of blood sample up to 2ml per withdrawal)

a) Restrain the animal and maintain the temperature around at 24 to 27°C.

b) Do not rub the tail from the base to the tip as it will result in leucocytosis. If the vein is not visible, the tail is dipped into warm water (40°C).

c) Apply local aesthetic cream on the surface of the tail 30 min before the experiment.

d) Insert a 23G needle into the blood vessel and blood is collected using a capillary tube or a syringe with a needle.

e) In case of difficulties, open the skin by giving cut of 0.5 to 1 cm and prick the vein with bleeding lancet or needle and collect blood with a capillary tube or a syringe with a needle.
f) After blood collection, apply pressure/silver nitrate ointment/solution to stop the bleeding.
g) Use temporary surgical cannula, if multiple samples are needed.

D. Tail snip blood sample collection (Recommended for blood collection only in mice)

a) This method should be avoided as far as possible because it can cause potential permanent damage on the animal tail. If needed, it should be done under terminal aesthesia only.
b) Apply local aesthesia, before collecting the blood, and make a cut of 1 mm from the tip of the tail using scalpel blade.
c) Stop the blood flow by dabbing the tail tip.

E. Orbital sinus blood sample collection

a) This technique is also called periorbital, posterior-orbital and orbital venous plexus bleeding.
b) Collect blood sample under general aesthesia.
c) Apply topical ophthalmic aesthetic agent to the eye before bleeding.
d) Scruff the animal with thumb and forefinger of the non-dominant hand and tightly pull the skin around the eye.
e) Insert a capillary into the medial canthus of the eye (30-degree angle to the nose). Slight thumb pressure is enough to puncture the tissue and enter the plexus/sinus.
f) Once the plexus/sinus is punctured, blood will come through the capillary tube.
g) Collect the required volume of blood from plexus, remove the capillary tube gently and wipe with sterile cotton.
h) Thirty minutes after blood collection, check animal for postoperative and periorbital lesions.

Caution

- Repeated blood sampling is not recommended.
- Skill is required to collect blood.
- Even a minor mistake will cause damage to the eye.
- Two weeks should be allowed between two bleedings.

F. Jugular vein blood sample collection

a) This method is used to collect micro volumes to one ml of blood sample.
b) Anesthetize the animal with suitable aesthetic.
c) Shave the neck region of the animal and keep in hyper extended position.
d) Clean the skin and sterilize using 70% alcohol.
e) Give a 3 cm incision on the back and create enough room in between skin and the body wall to locate the port.
f) The jugular veins appear blue in colon and is found 2 to 4 mm lateral to sternoclavicular junction.
g) Insert a 25G needle in the caudo cephalic direction (back to front) and slowly withdraw blood to avoid collapse of these small blood vessels. Handle animal carefully and take care not to insert needle more than 3 to 4 mm into the blood vessel.
h) If the attempt to collect blood fails, slowly remove the needle and monitor the site for bleeding. If there is no bleeding, one more attempt can be made. Avoid further attempts in case of bleeding as it may collapse the vein.
i) Finger pressure is applied to stop bleeding.

Caution

- Number of attempts is limited to three.

- Apply local anaesthetic cream 30 minutes prior to sampling.

G. Blood sample collection with temporary cannula

a) This method is used for a few hours by making a temporary cannulation in the tail vein.
b) Restrain the animal and apply local anaesthetic cream on the tail (1 – 2 cm above the tail tip).
c) Cannulate the tail or insert a 25G needle.
d) Tail bleeding normally requires the animal to be warmed in order to dilate the blood vessels (37 – 39°Cfor 5 – 15 min).
e) House the animal individually in large cage after cannulation.

Blood vessel cannulation

This method involves continuous and multiple sampling in the experimental animal and requires close and continuous monitoring of the animal. Usually, blood vessel cannulation is done in the femoral artery, femoral vein, carotid artery, jugular vein, vena cava and dorsal aorta. Surgery is required for this method and appropriate anaesthesia and analgesia should be used to minimize the pain. After surgical cannulation, animal should be housed singly in a large and spacious cage. Collect the blood sample of 0.1 to 0.2 ml, collection can be done over 24 hours.

After withdrawing the blood, flush the cannulas with an anticoagulant and replace the withdrawn volume (if required) with Lactated Ringer's solution and close the cannula tightly.

Caution: Carry out experiment fully under aseptic precautions. Infection, haemorrhage, blockage of cannula and swelling around the cannulation site should be looked for. The needle size and maximum blood volume to be collected are given in Table 1.

Species	**Needle to be used**	**Maximum collection volume**
Mice	23–24 G	1 ml
Rat	19 – 21 G	10 – 15 ml
Rabbit	19 – 21 G	60 – 200 ml
Guinea pig	20 – 21 G	1 – 25 ml

H. Tarsal vein blood sample collection

a) This method is commonly recommended for guinea pig.
b) Identify tarsal vein in one of the hind legs of animal.
c) Restrain the animal properly. Tarsal vein may be visible in blue colour.
d) Apply a suitable hair remover to remove the surface hairs.
e) Apply a local anaesthetic cream on the collection site.
f) Wait for 20 to 30 minutes and collect blood sample slowly by using 22G needle.
g) Take maximum three samples per leg of sample size 0.1 to 0.3 ml per sample.
h) After the sample collection, apply gentle pressure with finger for 2 minutes to stop bleeding.

Caution

- Not more than six samples from both hind legs are taken.
- The number of attempts is three or less.

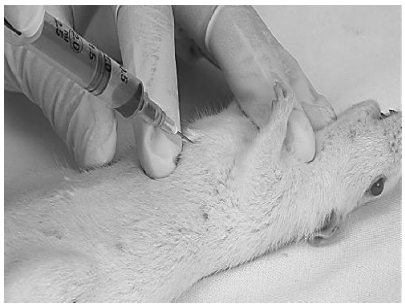
I. Marginal ear vein/artery blood sample collection

a) This method is commonly adopted for rabbits.
b) Place the animal in a restrainer.
c) Clean ear with 95% v/v alcohol and apply local anaesthetic cream on the collection site 10 min prior to sampling. (If required, apply o-Xylene/topical vasodilator topically on the collection site to dilate blood vessels).

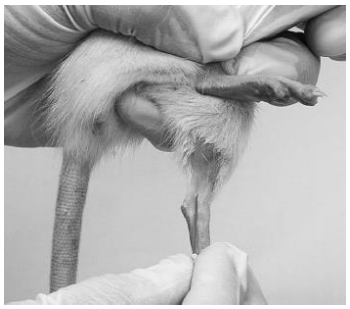
d) Give cut to the marginal ear vein by using surgical blade of size 11 and collect blood in a collecting tube. Otherwise, use a 26G needle to collect blood from animal marginal vein.
e) After collecting blood, cover the site with clean sterile cotton and apply gentle pressure to stop the bleeding.

J. Blood sample collection through posterior vena cava

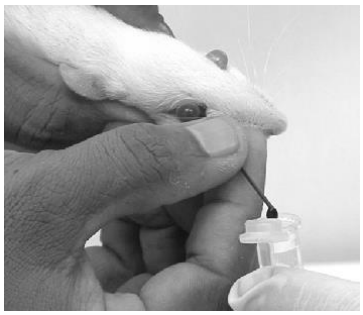
a) In general, posterior vena cava blood sample is recommended for terminal stage of the study.
b) Anesthetize animal and make a cut of 'Y'- or 'V'-shaped in the abdomen.
c) Remove the intestines gently.
d) Push the liver forward and identify the posterior vena cava (between the kidneys).
e) Insert 21 to 25G needle to collect blood from the posterior vena cava.
f) Repeat this procedure three to four times and collect more volume of blood sample.



Cardiac puncture



Saphenous vein



Retro-orbital plexus

9. Observations:

a. Complete the following table showing commonly recommended anaesthetic agents (along with doses) for laboratory animal experiments while collecting blood samples.

Sr. No.	Animal species	Anaesthesia
Mice		
Rats		
Guinea pig		
Rabbits		

b. Complete the following table by writing the outer diameter of the respective needle size.

Sr. No.	Needle size	Outer diameter (mm)
1.	12	
2.	15	
3.	18	
4.	21	
5.	24	
6.	26	

10. Result:

Various techniques of blood collection from animals along with sites were studied.

11. References:

a) Parasuraman S, Raveendran R, Kesavan R. Blood sample collection in small laboratory animals. JPharmacolPharmacotherap: 2010;1:87-93.

b) Paulose CS, Dakshina murti K. Chronic catheterization using vascular-accessport in rats: Blood sampling with minimal stress for plasma catecholamine determination. J Neurosci Methods1987;22:141-6.

c) Yo burn BC, Morales R, Inturrisi CE. Chronic vascular catheterization in the rat: Comparison of three techniques. PhysiolBehav1984;33:89-94.

d) mycalpharm.com by in fokart India Pvt. Ltd. &XploRemedies LLP.

12. Practical Related Questions (Teacher can give more questions to the student):

a) Name the method of blood collection for short experiments.

b) Give reason, why it is necessary to maintain aseptic environment while collecting blood from animals.

c) Which are the methods used to collect blood from the mice?

d) Give the uses of blood collection techniques.

e) If the experiment is carried out to test the anti-diabetic effects of drug; which method you should select to know the blood glucose level and comment on why?

(Space for answers)

13. Assessment Scheme:

Particular	Understanding the basic concept (Intellectual skill)	Activities (Intellectual and motor skill)	Cleanliness & Handling (Affective domain)	Viva-voce	Total	Signature of teacher
Marks obtained						
Max marks	**2**	**5**	**1**	**2**	**10**	

Experiment No. 8
Study of Local Anaesthetics on Rabbit Eye

1. Aim:

To study the effect of local anaesthetics on rabbit eye's cornea

2. Practical Significance:

Local anaesthetics are the agents that induce reversible loss of sensation locally in the parts of the body. These agents block the generation of action potential locally in the area where they are applied. They are used to perform minor surgeries. In this practical, the students will be able to identify and evaluate the effects of local anaesthetics on corneal reflex in rabbits.

3. Practical Outcomes (PrOs):

After completion of this practical, the students will be able to:

PrO	Practical Outcomes	Mapped CO	BTL
PrO1	Explain the mechanism of action of local anaesthetics.	CO1 &4	BTL2
PrO2	Discuss the effect of local anaesthetics on rabbit cornea.	CO1 & 4	BTL2
PrO3	Evaluate the effect of local anaesthetic on corneal reflex of animals.	CO1 & 4	BTL3
PrO4	Practice ethical behaviour and cleanliness in the laboratory	CO1 & 4	BTL5

4. Relevant Theoretical Background:

Local anaesthetics

Local anaesthetics (LAs) are the agents which induces loss of sensation reversibly by blocking impulse conduction along nerve axons and in excitable membranes. This action is prominent in the neurons and excitable membranes that primarily utilizes Na+ channels as means for generation of action potential.

LAs block the Na+ channels causing blockade of initiation and propagation of action potential. The examples of local anaesthetic agents are: cocaine, procaine, tetracaine, benzocaine, lidocaine, mepivacaine, prilocaine, bupivacaine, etidocaine, etc. Procaine has poor penetration and hence not used as a surface anaesthetic. Cocaine produces corneal sloughing (protoplasmic poison) and hence not used.

LAs are used to produce

a. Surface anaesthesia: It is the application of an LA to the skin or a mucous membrane.

b. Infiltration anaesthesia: It is infiltration of LA into the tissue to be anesthetized; surface and infiltration anaesthesia are collectively called as topical anaesthesia.

c. Field block: It is subcutaneous injection of an LA in an area bordering on the field to be anesthetized.

d. Peripheral nerve block: It is injection of LA in the vicinity of a peripheral nerve to anesthetize that nerve's area of innervations.

e. Epidural anaesthesia: It is an LA injected into the epidural space, where it acts primarily on the spinal nerve roots.

f. Spinal anaesthesia: It is an LA injected into the cerebrospinal fluid, usually at the lumbar spine (in the lower back), where it acts on spinal nerve roots and part of the spinal cord.

Corneal reflex

A fine cotton wool wick is used to test the corneal reflex. The wick is made in such a way that there are no distended cotton parts. With the tip of a cotton wick, gently touch the cornea's peripheral sides.

Always bring the cotton wool forward from the side (back). Cotton wool or your hand should not be shown to the rabbit. The presence of a corneal reflex is indicated by blinking. LAs block corneal reflex in the rabbit eye.

5. Precaution:

Do not touch central part of cornea it can cause corneal ulcers/opacities. This can lead to blindness as central part of cornea is the main part of cornea used for visibility.

6. Resources required:

Rabbit, scissor, dropper, cotton, saline solution and drug (xylocaine 1-2% or lignocaine 1-2%)
Watch the video of "Study of local anaesthetics on rabbit eye" using the YouTube video or any other video/software/MSBTE CD available.

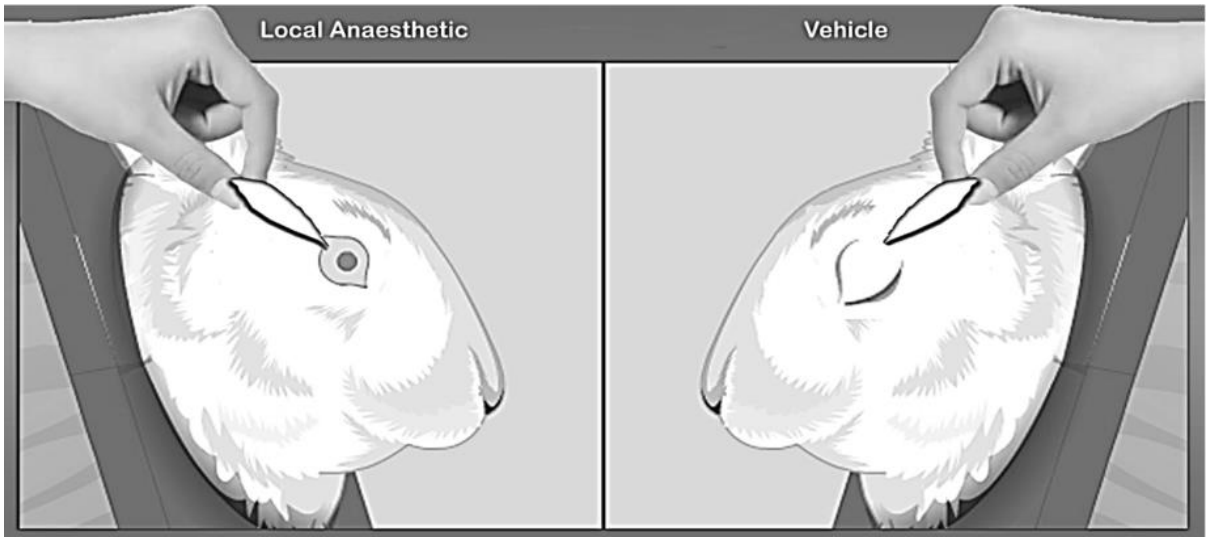
7. Resources used:

_____

_____

8. Procedure:

a. Select the healthy rabbit weighing 2-3 kg.
b. Handle rabbit with care and restrain it using rabbit restrainer.
c. Clip off eyelashes of both the eyes or cut the eyelashes of both eyes with scissor.
d. Use pouch method to instil drugs into eye. Pinch lower eyelid to make a small pouch.
e. Instil 1-2 drops of saline/drug in it using dropper. Pull the lower eyelid upwards and keep it in contact with conjunctiva for 1-2 minutes.
f. You can also press medial canthus for 5 seconds after instillation of drug.
g. Keep one eye (either right or left eye) as control and the other as test.
h. Apply saline in the control eye and a drug (xylocaine) in the test eye.
i. Start the stop watch to record the timings.
j. With the tip of a cotton wick, gently touch the cornea's peripheral sides.
k. Observe the corneal reflex (blinking of eye) on instillation of drug after 1 minute.
l. Observe the corneal reflex till the rabbit recovers from the drug effect.
m. Note down the observation in the observation table.
n. Take 3 readings to confirm recovery from the drug effect.



9. Observations:

Record the observation from the software in the following table. Write + and - for the presence and absence of corneal reflex respectively.

Time (min)	Corneal reflex	
	Left eye (Xylocaine 1%)	**Right eye (Saline)**
1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		
9.		
10.		
11.		
12.		
13.		
14.		
15.		

10. Result:

a. Onset of action of xylocaine (absence of corneal reflex) was found to be _____ min.

b. Duration of action of xylocaine (time duration from loss of corneal reflex to recovery) was found to be_____ min.

11. Conclusion:

Xylocaine was found to produce local anesthetic effect.

12. References:

a) Kulkarni SK. Practical Pharmacology and Clinical Pharmacy. First edition, Vallabh Prakashan, Delhi.

b) mycalpharm.com by in fokart India Pvt. Ltd. & Xplo Remedies LLP.

13. Practical Related Questions (Teacher can give more questions to the student):

a) Explain the generation of action potential.

b) Describe the mechanism of local anaesthetics.

c) Give the classification of local anaesthetics with examples.

d) Write indications of local anaesthetics.

e) Give the various types of local anesthesia along with their indications.

f) Illustrate surface anesthesia and membrane stabilizing effect.

(Space for answers)

14. Assessment Scheme

Particular	Understanding the basic concept (Intellectual skill)	Activities (Intellectual and motor skill)	Cleanliness & Handling (Affective domain)	Viva-voce	Total	Signature of teacher
Marks obtained						
Max marks	**2**	**5**	**1**	**2**	**10**	

Experiment No. 9
Study of Mydriatic Effect on Rabbit Eye

1. Aim:

To study the effect of Mydriatics on rabbit eye

2. Practical Significance:

Mydriatics cause dilation of pupil. They are used to reduce painful ciliary muscle spasm, for diagnostic purposes to allow for more detailed examination of the retina and other structures deep in the eye, for treating chronic simple glaucoma, for treating corneal ulcers, for diagnosing Horner's syndrome (caused by the disruption of a nerve pathway from the brain to the face and eye on one side of the body), for surgery of cataract, etc. In this practical, the students will be able to identify and evaluate the effects of mydriatics on rabbit's cornea.

3. Practical Outcomes (PrOs):

After completion of this practical, the students will be able to:

PrO	Practical Outcomes	Mapped CO	BTL
PrO1	Explain the mechanism of action of mydriatics.	CO1	BTL2
PrO2	Discuss the effect of mydriatics on the pupil size, corneal and light reflex.	CO1	BTL2
PrO3	Evaluate the effect of mydriatics on the pupil size, corneal and light reflex.	CO1	BTL5
PrO4	Practice ethical behaviour and cleanliness in the laboratory	CO1	BTL5

4. Relevant Theoretical Background:

Mydriatics

The eye is supplied with both sympathetic and parasympathetic autonomic nerves. The sympathetic innervation is present on superior palpebral muscle and the dilator pupillae (circular fibers) of the iris, whereas parasympathetic innervation is present on ciliary muscles and sphincter pupillae (radial fibers) of the iris. Out of these, the parasympathetic innervation has dominant actions. Bright light causes contraction of circular muscles that results in miosis, whereas dim light causes contraction of radial muscles results in dilation of pupil (see figure). Parasympathetic stimulation causes ciliary muscle to contract and move the ciliary body inwards and forwards. This makes lens to bulge forward and the eye is accommodated for near vision. Opposite happens when ciliary muscle relaxation occurs resulting in paralysis of accommodation (cycloplegia). The intraocular pressure is increased when sympathetic stimulation contracts the dilator pupillae that dilates the pupil, the iris folds back near the opening of the canal of schlem and the drainage of aqueous humous is decreased. Opposite happens when the pupil constricts that increases drainage and reduces intraocular pressure.

Mydriatics are the agents that causes dilation of the pupil. The examples of mydriatics are: adrenaline, ephedrine, cyclopentolate, tropicamide, and atropine, etc. Mydriatics like adrenaline causes mydriasis because of contraction of radial muscle fibers (dilator pupillae) and relaxation of circular muscle fibers(constrictor pupillae) of iris of eye due to sympathetic stimulation. While parasympatholytic like atropine induces mydriasis by blocking contraction of the circular pupillary sphincter muscle, which is normally stimulated by acetylcholine release, thereby allowing the radial iris dilator muscle to contract and dilate the pupil.

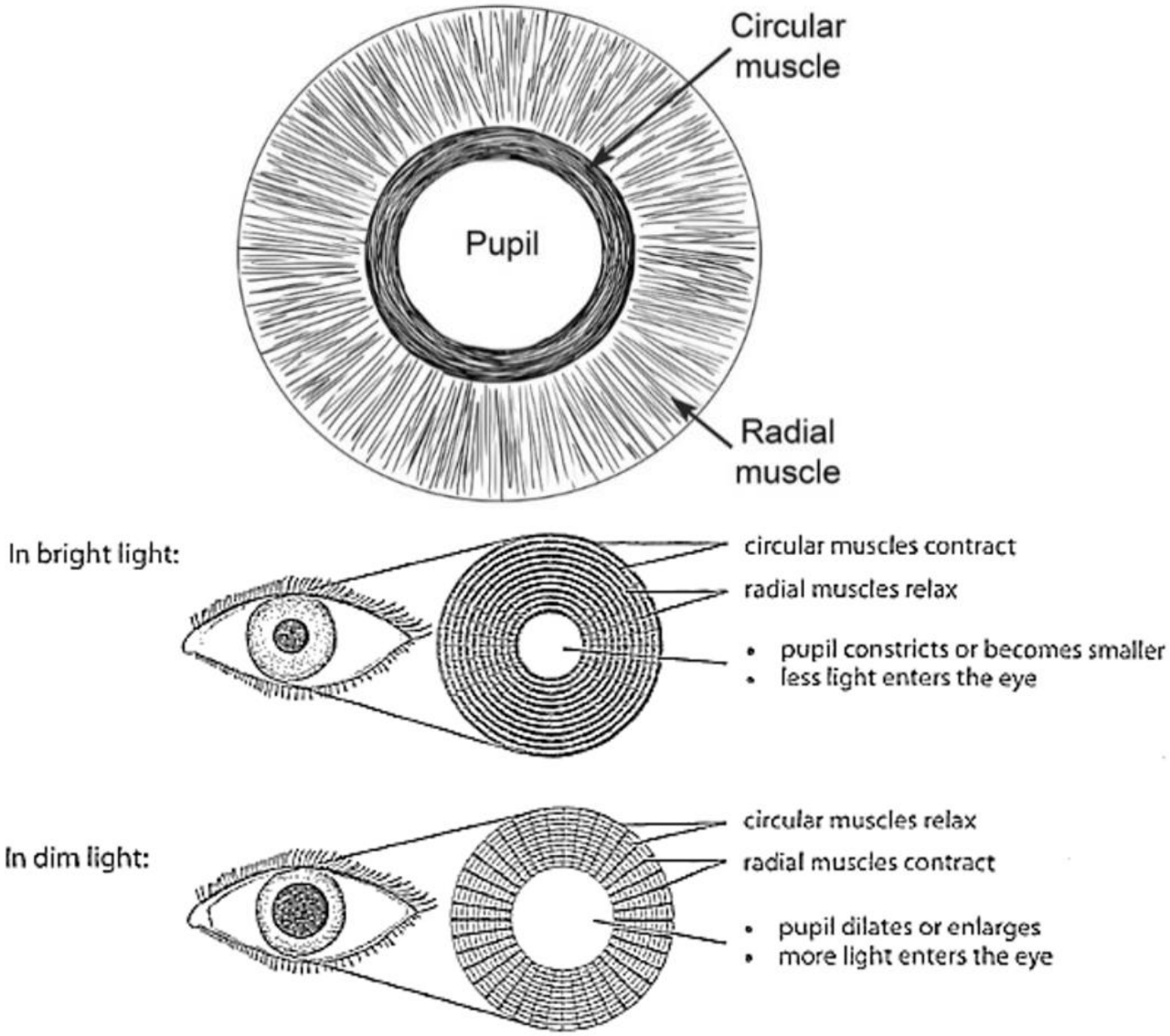


Figure 9.1 Effect of light reflex on rabbit's eye

Pupil size

A transparent measuring plastic scale is used to measure the diameter of the pupil by placing it in front of the eye as close as possible.

Light reflex

Light reflex is observed by putting the light from side and then to front (to and fro).

Corneal reflex

A fine cotton wool wick is used to test the corneal reflex. The wick is made in such a way that there are no distended cotton parts. With the tip of a cotton wick, gently touch the cornea's peripheral sides.

Always bring the cotton wool forward from the side (back). Cotton wool or your hand should not be shown to the rabbit. The presence of a corneal reflex is indicated by blinking.

5. Precaution:

- Do not put the scale on the cornea to measure pupil size, but it should be close as possible.
- Do not touch central part of cornea it can cause corneal ulcers/opacities. This can lead to blindness as central part of cornea is the main part of cornea used for visibility.

6. Resources required:

Rabbit, rabbit holder, scissor, dropper, cotton, transparent measuring scale, torch, saline solution and drug (atropine 1% and phenylephrine 20%)

Watch the video of "Study of Mydriatic effect on rabbit eye" using the YouTube video or any other video/software/MSBTE CD available.

7. Resources used:

_____

_____

8. Procedure:

a. Select the healthy rabbit weighing 2-3 kg.
b. Handle rabbit with care and restrain it using rabbit restrainer or put it in rabbit holder.
c. Clip off eyelashes of both the eyes or cut the eyelashes of both eyes with scissor.
d. Note down the size of the pupil of both eyes.
e. Observe the light reflex by putting the light from the side then to the front (to and fro).
f. Observe the corneal reflex by touching the side of the cornea with cotton wick.
g. Use pouch method to instil drugs into eye. Pinch lower eyelid to make a small pouch.
h. Instil 1-2 drops of saline to the left eye and consider it as control eye.
i. Instil 1-2 drops of drug in right eye and consider it as test eye.
j. After adding the drops, press the medial canthus for 10 seconds.
k. Record the diameter of pupil, presence or absence of light reflex and presence or absence of corneal reflex after 1, 5, 10, and 15 minutes.
l. Note down the observations in the observation table.
m. Take 3 readings to confirm the recovery from the effect of the mydriatic drug.

9. Observations:

Record the observation from the software/CD in the following table. Observe and record the size of pupil as normal, increased or decreased diameter of pupil. Write + and – for the presence and absence of light reflex or corneal reflex respectively.

Time after drug instillation	Drug	Diameter of pupil	Light reflex	Corneal reflex
1 minute	Saline	Normal	+	+
	Atropine			
	Phenylephrine			
5 minute	Saline			
	Atropine			
	Phenylephrine			
10 minute	Saline			
	Atropine			
	Phenylephrine			
15 minute	Saline			
	Atropine			
	Phenylephrine			

10. Result:

Atropine and/or Phenylephrine were found to produce mydriasis.

11. Conclusion:

Atropine and/or Phenylephrine possess mydriatic properties.

12. References:

a) Kulkarni SK. Practical Pharmacology and Clinical Pharmacy. 1st edition, Vallabh Prakashan, Delhi.

b) Badyal D. Practical manual pharmacology. 1st edition, Jaypee Brothers Medical Publishers, New Delhi.

c) Mycalpharm.com by infokart India Pvt. Ltd. &XploRemedies LLP.

13. Practical Related Questions (Teacher can give more questions to the student):

a) Explain the sympathetic activity on the eye.

b) Describe the effects of atropine on eye.

c) Classify the parasympatholytics with examples.

d) Write indications of mydriatics.

e) Explain Horner's syndrome.

f) Write the actions of acetylcholine esterase on acetylcholine.

(Space for answers)

14. Assessment Scheme

Particular	Understanding the basic concept (Intellectual skill)	Activities (Intellectual and motor skill)	Cleanliness & Handling (Affective domain)	Viva-voce	Total	Signature of teacher
Marks obtained						
Max marks	**2**	**5**	**1**	**2**	**10**	

Experiment No. 10

Study of **Miotic** Effect on Rabbit Eye

1. Aim:

To study the effect of miotics on rabbit's cornea.

2. Practical Significance:

Miotics cause constriction of pupil. They are used to diagnose the pupil abnormalities (e.g., Adie's tonic pupil) and used in the treatment of ocular hypertension, acute angle closure glaucoma, dry eye and post-surgical glare. Parasympathomimetic drugs cause smooth muscle contraction of the iris sphincter and ciliary body. This leads to the contraction of the iris sphincter causing the pupil to decrease in size, and the contraction of the ciliary body opens the trabecular meshwork, decreasing intraocular pressure by enhancing aqueous out flow. In this practical, the students will be able to identify and evaluate the effects of miotics on rabbit's cornea.

3. Practical Outcomes (PrOs):

After completion of this practical, the students will be able to:

PrO	Practical Outcomes	Mapped CO	BTL
PrO1	Explain the mechanism of action of miotics.	CO1	BTL2
PrO2	Discuss the effect of miotics on the pupil size, corneal and light reflex.	CO1	BTL2
PrO3	Evaluate the effect of miotics on the pupil size, corneal and light reflex.	CO1	BTL5
PrO4	Practice ethical behaviour and cleanliness in the laboratory	CO1	BTL5

4. Relevant Theoretical Background:

Miotics

The eye is supplied with both sympathetic and parasympathetic autonomic nerves. The sympathetic innervation is present on superior palpebral muscle and the dilator pupillae (circular fibers) of the iris, whereas parasympathetic innervation is present on ciliary muscles and sphincter pupillae (radial fibers) of the iris. Out of these the parasympathetic innervation has dominant actions. Bright light causes contraction of circular muscles that result in miosis, whereas dim light causes contraction of radial muscles results in dilation of pupil. Parasympathetic stimulation causes ciliary muscle to contract and move the ciliary body inwards and forwards. This makes lens to bulge forward, and the eye is accommodated for near vision.

Opposite happens when ciliary muscle relaxation occurs resulting in paralysis of accommodation (cycloplegia). The intraocular pressure is increased when sympathetic stimulation contracts the dilator pupillae that dilates the pupil, the iris folds back near the opening of the canal of schlemm and the drainage of aqueous humous is decreased. Opposite happens when the pupil constricts that increases drainage and reduces intraocular pressure.

Miotics are the agents that cause constriction of the pupil. The examples of miotics are: physostigmine, pilocarpine, ecothiopate, etc. Miotics like physostigmine causes miosis because of contraction of circular muscle fibers (constrictor pupillae) and relaxation of radial muscle fibers (dilator pupillae) of iris of eye due to parasympathetic stimulation.

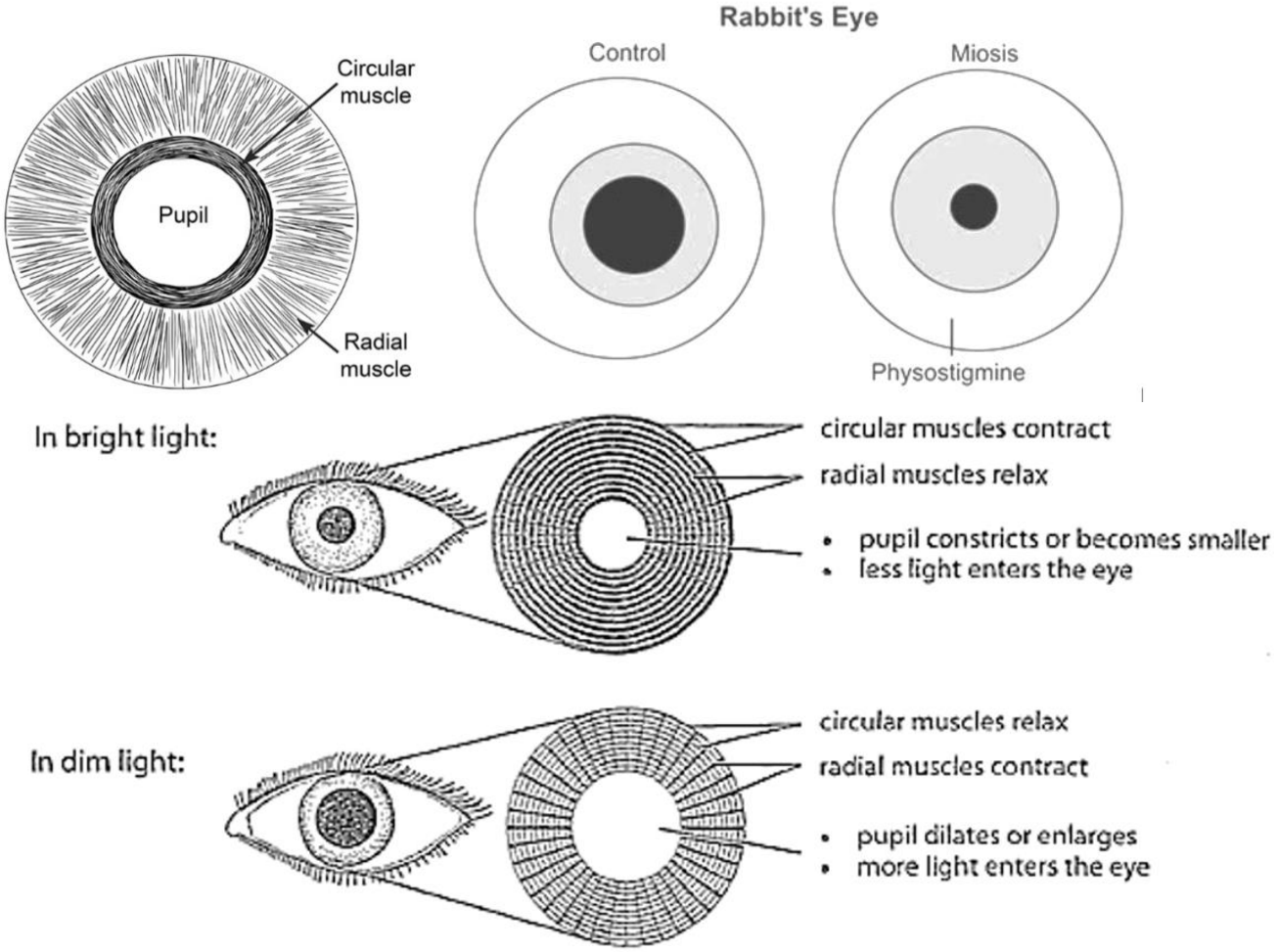


Figure 10.1 Effect of physostigmine and light reflux on rabbit's eye

Pupil size

A transparent measuring plastic scale is used to measure the diameter of the pupil by placing it in front of the eye as close as possible.

Light reflex

Light reflex is observed by putting the light from side and then to front (to and fro).

Corneal reflex

A fine cotton wool wick is used to test the corneal reflex. The wick is made in such a way that there are no distended cotton parts. With the tip of a cotton wick, gently touch the cornea's peripheral sides.

Always bring the cotton wool forward from the side (back). Cotton wool or your hand should not be shown to the rabbit. The presence of a corneal reflex is indicated by blinking.

5. Precaution:

- Do not put the scale on the cornea to measure pupil size, but it should be as close as possible.
- Do not touch central part of cornea, it can cause corneal ulcers/opacities. This can lead to blindness as central part of cornea is the main part of cornea used for visibility.

6. Resources required:

Rabbit, rabbit holder, scissor, dropper, cotton, transparent measuring scale, torch, saline solution and drug (physostigmine 1% and pilocarpine 4%)

Watch the video of "Study of Miotic effect on rabbit eye" using the YouTube video or any other video/software/MSBTE CD available.

7. Resources used:

_____

_____

8. Procedure:

a. Select the healthy rabbit weighing 2-3 kg.
b. Handle rabbit with care and restrain it using rabbit restrainer or put it in rabbit holder.
c. Clip off eyelashes of both the eyes or cut the eyelashes of both eyes with scissor.
d. Note down the size of the pupil of both eyes.
e. Observe the light reflex by putting the light from the side then to the front (to and fro).
f. Observe the corneal reflex by touching the side of the cornea with cotton wick.
g. Use pouch method to instil drugs into eye. Pinch lower eyelid to make a small pouch.
h. Instil 1-2 drops of saline to the left eye and consider it as control eye.
i. Instil 1-2 drops of drug in right eye and consider it as test eye.
j. After adding the drops, press the medial canthus for 10 seconds.
k. Record the diameter of pupil, presence or absence of light reflex and presence or absence of corneal reflex after 1, 5, 10, and 15 minutes.
l. Note down the observation in the observation table.
m. Take 3 readings to confirm the recovery from the effect of the miotic drug.

9. Observations:

Record the observation from the software in the following table. Observe and record the size of pupil as normal, increased or decreased diameter of pupil. Write + and – for the presence and absence of light reflex or corneal reflex respectively.

Time after drug instillation	Drug	Diameter of pupil	Light reflex	Corneal reflex
1 minute	Saline	Normal	+	+
	Physostigmine			
	Pilocarpine			
5 minute	Saline			
	Physostigmine			
	Pilocarpine			
10 minute	Saline			
	Physostigmine			
	Pilocarpine			
15 minute	Saline			
	Physostigmine			
	Pilocarpine			

10. Result:

Pilocarpine and/or Physostigmine was found to produce miotic effect.

11. Conclusion:

Pilocarpine and/or Physostigmine possesses miotic properties.

12. References:

a) Kulkarni SK. Practical Pharmacology and Clinical Pharmacy. 1st edition, Vallabh Prakashan, Delhi.
b) Badyal D. Practical manual pharmacology. 1st edition, Jaypee Brothers Medical Publishers, New Delhi.
c) mycalpharm.com by infokart India Pvt. Ltd. &XploRemedies LLP.

13. Practical Related Questions (Teacher can give more questions to the student):

a) Explain the parasympathetic activity on the eye.
b) Whether selective α-2 adrenergic agonists are used as miotics, why?
c) Write examples of sympathomimetics used as miotics.
d) Write indications of miotics.
e) Explain glaucoma.

(Space for answers)

14. Assessment Scheme:

Particular	Understanding the basic concept (Intellectual skill)	Activities (Intellectual and motor skill)	Cleanliness & Handling (Affective domain)	Viva-voce	Total	Signature of teacher
Marks obtained						
Max marks	**2**	**5**	**1**	**2**	**10**	

Experiment No. 11

Pyrogen Testing by Rabbit Method

1. Aim:

To study the presence of pyrogen in given parenteral solution using rabbit

2. Practical Significance:

Pyrogen is an endotoxin, chemically lipopolysaccharides, when introduced systemically causes increase in body temperature, chills, body ache, cutaneous vasoconstriction, rise in arterial blood pressure and even death. It is the waste product of metabolism of gram-negative bacteria like Escherichia coli, Proteus, Pseudomonas, Enterobacter, and Klebsiella. Once they are present in parenteral solution, they cannot be removed by any method and preparation has to be discarded. Hence, we have to ensure that parenteral preparations are pyrogen free. Rabbits are more sensitive to the pyrogens, gives the reproducible results and the tests are economical; hence they are used for the study. In this practical, the students will be able to identify and evaluate the presence of pyrogens in parenteral products using rabbit method.

3. Practical outcomes (PrOs):

After completion of this practical, the students will be able to:

PrO	Practical Outcomes	Mapped CO	BTL
PrO1	Explain the method of pyrogen testing in rabbits.	CO4	BTL2
PrO2	Interpret the results of pyrogen testing.	CO4	BTL4
PrO3	Record the body temperature of rabbit.	CO4	BTL3
PrO4	Practice ethical behaviour and cleanliness in the laboratory	CO4	BTL5

4. Relevant Theoretical Background:

Pyrexia

The increase in body temperature than the normal is called pyrexia (hyperthermia). The pyrogens suppress heat-sensing neurons and activate cold-sensing neurons. The temperature regulating centre in the hypothalamus sense this cooler temperature and responds by raising the body's temperature over normal, resulting in a fever. The prostaglandins released due to inflammation or injury may also increase body temperature.

Pyrogens

Pyrogens are the fever inducing organic substances (metabolic products of microorganisms) responsible for many febrile reactions. Pyrogens are endotoxins or lipopolysaccharide and when present systemically in sufficient quantity can lead to severe signs of inflammation, shock, multi organ failure, and sometimes even death in humans. Chemically pyrogens are lipids comprises of complex polysaccharide or polypeptide molecules containing phosphorous and nitrogen.

Due to the severe consequences of an infection, an injectable healthcare product such as a vaccine or intravenous solution must be sterile or free of live bacteria, but the manufacturing process to kill any bacteria can result in release of LPS or endotoxin into the product. Just as with a bacterial infection or sepsis, if sufficient endotoxin gets into our blood stream or spinal fluid we can develop fever, shock, and organ failure. In extreme cases, it can even result in death.

Therefore, injectable or implantable, products that come in contact with the blood stream or spinal fluid are tested for sterility (the absence of live bacteria) as well as endotoxin. Testing for endotoxin helps assure product quality and safety.

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 generates reproducible results that are similar to threshold response to humans and also economic.

The pyrogen test aims to check the existence of pyrogens by using rabbits. The pyrogen test is based on the measurement of the increase in the rabbit's temperature upon being injected with a product that might contain a contaminant of the pyrogen type.

Limulus amebocyte lysate (LAL) test is an alternative to rabbit pyrogen test. It is based on the principle that pyrogens when added to a suitable LAL preparation, causes gelation or increase in viscosity of that lysate which is directly proportional to the amount of endotoxin or pyrogen. This test requires less time, less amount of test sample and rules out biological variations.

Telethermometer

Telethermometer consist of a digital counter, rotating knob, six probes and sockets for measurment of temperature. This equipment has been calibrated to provide a 0.1^0C accuracy . To measure the temprature of rabbit, a probe that measures temperature is introduced into the test rabbit's rectum up to a depth of around 5 cm.

Figure 11.1 Digital Telethermometer

5. Precautions:

- Acclimatize the rabbits to the experimental conditions for 1 to 4 days before the start of experiment.
- Select the healthy rabbit that does not have fluctuations in their body temperature reading.
- Do not select the rabbit if their body temperature is more than 49.8 ˚C or they have been used for the pyrogen testing during last two weeks.
- The temperature sensing probe should be inserted in the rectum about 5 cm depth to record correct body temperature.
- Carefully and aseptically inject the testing solution, as it may affect the results.

6. Resources required:

Rabbit (healthy weighs up to 1.5 kg and fed on complete and healthy diet), rabbit holder, telethermometer, weighing balance, stop watch, cotton, syringe, saline solution and parenteral preparation to be tested.

Watch the video of "Pyrogen testing by rabbit method" using the YouTube video or any other video/software/MSBTE CD available.

7. Resources used:

_____

_____

8. Procedure:

As per I. P. the pyrogen test on rabbit is carried in two steps as preliminary test (SHAM test) and main test.

A. Preliminary test (SHAM test)

a. If rabbits are first time used for pyrogen test or they are not used for experiment before two weeks then conditioning of rabbits is done for 1 to 3 days before the testing of the parenteral products.

b. Clean all the glassware, syringes, and needles thoroughly with water for injection and place it in hot air oven for 30 min at 250 ˚C or for 1 hour at 200 ˚C.

c. Place rabbits in rabbit holder and start to record the body temperature at intervals of 30 min by inserting the temperature sensing probe in to the rabbit's rectum.

d. Note down the stable reading form the digital thermometer.

e. After three consecutive readings (after 90 min), administer the pyrogen free saline solution warmed at38.5 ˚C in the marginal vein of the rabbit's pinna.

f. Record the body temperature at 30 min intervals for consecutive 3 hours.

g. If any rabbit shows the deflection of the temperature by 0.6 ˚C during observation for 3 hours, should not be selected for the main test.

B. Main test

a. Choose the 3 rabbits selected in Sham's test and weigh them.

b. Calculate the dose as 10 ml/kg of body weight.

c. Restrain the rabbit by keeping them in rabbit holder, they should be there for the whole test period.

d. Insert the temperature sensing probe or the thermometer into the rectum at the depth of 5 cm to record the body temperature.

e. Record the temperature at the intervals of the 30 min, beginning 90 min before injecting the test solution.

f. Warm the solution at 38.5 ˚C before injecting.

g. Inject the test solution slowly into the marginal vein of ear over a period of 4 min.

h. Record the temperature of each rabbit at 30 min intervals for 3 hours.

i. Calculate the average of the two temperature reading at 60th min and 90th min, consider it as initial temperature.

j. The highest temperature recorded for rabbit after the injection (i.e., in between 120 min to 270 min) is referred as maximal temperature.

k. Calculate the difference between maximal and initial temperature. This difference should be recorded as response (when the difference is negative then record it as zero).

Interpretation of Result

1. If the sum of responses of group of three rabbits does not exceeds 1.4˚C or if the response of individual rabbit is less than 0.6˚C, then the given sample passes the test.

2. If the response of any rabbit is 0.6˚C or more or if the sum of the responses of the three rabbits exceeds1.4˚C, then continue the test using 5 other rabbits.

3. If not more than three of the eight rabbits show individual responses of 0.6˚C or more and if the sum of the responses of the group of eight rabbits does not exceed 3.7˚C, then the given sample passes the test for pyrogen.

9. Observations:

Record the observation from the software in the following table.

Parameters	**Time (min)**	**Temperature reading (˚C)**		
		Rabbit 1	**Rabbit 2**	**Rabbit 3**
Initial body temperature	0			
Body temperature after Injection	30			
	60			
	90			
	120			
	150			
	180			
Difference in body temperature = (highest temperature after injection - initial temperature before injection)				
Sum of difference in body temperature				

- If the rise in temperature of any rabbit is 0.6 ˚C or more or if the sum of responses of three rabbits exceeds 1.4˚C then select 5 more rabbits and continue the test.

10. Results (fill that is applicable):

a. The individual responses of three rabbits were: _____ ˚C, ____ ˚C, and ____ ˚C.

b. The sum of the differences in body temperature of three rabbits was: _____ ˚C.

11. Conclusion:

From the results it can be concluded that the given sample _____ (passes /not passes) the test for pyrogen as per IP 2010. Hence, it is _____ (suitable/not suitable) for use.

12. References:

a) Kulkarni SK. Practical Pharmacology and Clinical Pharmacy. 1st edition, Vallabh Prakashan, Delhi.

b) Badyal D. Practical manual pharmacology. 1st edition, Jaypee Brothers Medical Publishers, New Delhi.

c) mycalpharm.com by infokart India Pvt. Ltd. &XploRemedies LLP.

13. Practical Related Questions (Teacher can give more questions to the student):

a) State whether pyrogens are water soluble or not?

b) State the process of de pyrogenation and inactivation of pyrogens.

c) Define endotoxins.

d) Why the pyrogen test is replaced by LAL test in IP 2018?

e) Name the parts of telethermometer.

f) Define antipyretics and give three examples of it.

(Space for answers)

14. Assessment Scheme

Particular	Understanding the basic concept (Intellectual skill)	Activities (Intellectual and motor skill)	Cleanliness & Handling (Affective domain)	Viva-voce	Total	Signature of teacher
Marks obtained						
Max marks	**2**	**5**	**1**	**2**	**10**	

Experiment No. 12

Study of Analgesic Activity by Tail Flick Method

1. Aim:

To study the effect of analgesics using tail flick apparatus

2. Practical Significance:

Analgesia is the term used to describe the reduced alertness to pain. The analgesics or antinociceptive agents decrease sensation of pain by increasing the threshold to pain stimuli. A noxious stimulus is used to induce painful reaction in animals such as thermal (radiant heat, high temperature), chemical (acetic acid, bradykinin) or physical pressure (tail compression). In this practical, the students will be able to understand the effects of analgesics by increasing pain threshold and evaluating the antinociceptive actions of drugs using tail-flick apparatus that induces pain through radiant heat.

3. Practical outcomes (PrOs):

After completion of this practical, the students will be able to:

PrO	Practical Outcomes	Mapped CO	BTL
PrO1	Explain the mechanism of action of analgesics.	CO2	BTL2
PrO2	Describe the use of tail-flick apparatus in evaluation of analgesic drugs.	CO2	BTL2
PrO3	Handle the software for recording the responses.	CO2	BTL3
PrO4	Practice ethical behaviour and cleanliness in the laboratory	CO2	BTL5

4. Relevant Theoretical Background:

Analgesia

Desensitization to pain without loss of consciousness is called as analgesia. The substance that produces this response is called as analgesics and they produce their action by increasing the threshold for the pain stimuli. The commonly used analgesics are morphine (narcotic analgesics) and aspirin, paracetamol (non-narcotic analgesics).

Pain receptors (Nociceptors), can be excited by three types of stimuli: mechanical, thermal, and chemical. Chemical substances produced by the body that excite pain receptors include bradykinin, serotonin, and histamine. Prostaglandins are fatty acids that are released when inflammation occurs and can increase the pain sensation by sensitizing the nerve endings; that increase in sensitivity is called hyperalgesia.

Opioids are potent pain-relieving medications and are used to treat severe pain. Morphine, an extremely effective analgesic acts by mimicking the endorphins produced naturally by the body by binding to their receptors and blocking or reducing the activation of pain neurons.

The non-narcotic anti-inflammatory analgesics like aspirin, NSAIDs, and COX inhibitors either non selectively or selectively block the activity of COX enzymes. COX enzymes are responsible for the conversion of arachidonic acid (a fatty acid) to prostaglandins, which enhance sensitivity to pain.

Tail-flick Method

Tail-flick method is one of the most common tests based on a phasic stimulus of high intensity. It works on the radiant heat-induced noxious stimulus to create pain. The radiant heat is applied to a small area of the tail and reaction time to remove the tail within 3-5 sec from this stimulus is observed. A cut off period of 10-12 sec is observed to prevent damage to tail. If the drug increases the threshold to painful stimulus then there will be increase in basal reaction time. It was first described by D'Amour and Smith in 1941.

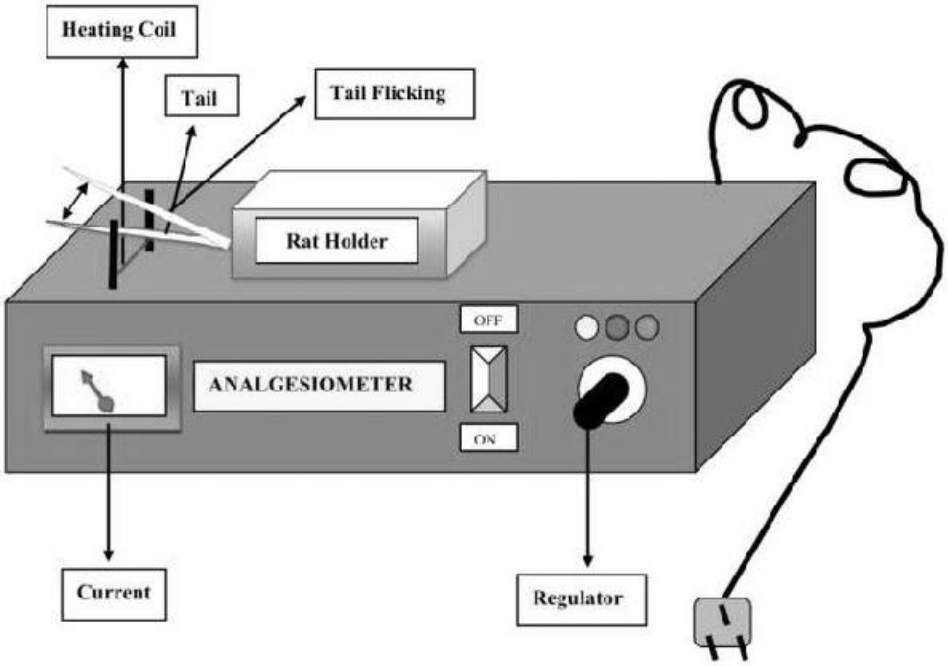


Figure 12.1 Tail-flick apparatus

5. Precautions:

a. The tail-flick method is simple to perform but animals should be accustomed to being handled so that they remain calm during measurements.

b. Do not select the animal if it fails to withdraw tail after 4 sec (without treatment).

c. The cut off time of 15 seconds is taken as maximum analgesic response to avoid damage to the tail due to heat.

6. Resources required:

Animal: Mice (20-25 g)

Drugs: Morphine sulphate (Dose- 5 mg/kg, S.C., prepare a stock solution containing 0.5 mg/ml and inject1 ml/100 g of body weight of mouse)

Apparatus: Tail-flick apparatus

Watch the video of "Study of Analgesic Effect using Tail Flick Method" using the YouTube video or any other video/software/MSBTE CD available.

7. Resources used:

_____

_____

8. Procedure:

a. Weigh and number the animals.

b. Record basal reaction time: Place the tip of the tail on the radiant heat source (hot wire) and the tail-flicking response is taken as the end point. Note down the time required to flick the tail. Normally a mouse withdraws its tail within 3-5 sec.

c. Any animal failing to withdraw its tail in 3-5 sec. should be rejected from the test.

d. Confirm the normal behaviour: Take near about 5 basal readings for individual mouse at the gap of 5 min.

e. Inject the drugs like morphine s.c. and note the reaction time at a 5, 15, 30, and 60 min after the drug.

f. Cut off time: As the reaction time reaches 15 seconds it is considered maximal analgesia and the tail is removed from the source of heat to avoid tissue damage.

g. Calculate highest percentage increase in reaction time as index of analgesia at each time interval for individual mouse.

9. Observations:

Record the observation from the software in the following table:

Sr. no.	**Basal reaction time (sec)**					**Reaction time (sec) after drug administration**			
	1	2	3	4	5	5 min	15 min	30 min	60 min
1									
2									
3									
4									
5									
6									

10. Result:

The reaction time was found to be _____ (increased/decreased).

11. Conclusion:

From the result it can be concluded that the given drugs _____ (possess / does not possess)the analgesic activity.

12. References:

a) Kulkarni SK. Practical Pharmacology and Clinical Pharmacy. First Edition, Vallabh Prakashan, Delhi.

b) MasoumeRezaee-Asl, MandanaSabour, VahidNikoui, Sattar Ostadhadi, AzamBakhtiarian, "TheStudy of Analgesic Effects of Leonuruscardiaca L. in Mice by Formalin, Tail Flick and Hot Plate Tests",International Scholarly Research Notices, vol. 2014, Article ID 687697, 5 pages, 2014.

c) mycalpharm.com by infokart India Pvt. Ltd. &XploRemedies LLP.

13. Practical Related Questions (Teacher can give more questions to the student):

a) Explain the physiology of pain sensation in brief.

b) Define narcotic and non-narcotic analgesics with examples.

c) Why to follow the cut off time in test?

d) Write the mechanism of action of morphine.

(Space for answers)

14. Assessment Scheme:

Particular	Understanding the basic concept (Intellectual skill)	Activities (Intellectual and motor skill)	Cleanliness & Handling (Affective domain)	Viva-voce	Total	Signature of teacher
Marks obtained						
Max marks	**2**	**5**	**1**	**2**	**10**	

Experiment No. 13

Study of Analgesics by Hot Plate Method

1. Aim:

To study the effect of analgesics using hot plate apparatus

2. Practical Significance:

Analgesia is the term used to describe the reduced alertness to pain. The analgesics or antinociceptive agents decrease sensation to pain by increasing the threshold to pain stimuli. A noxious stimulus is used to induce painful reaction in animals such as thermal (radiant heat, high temperature), chemical (acetic acid, bradykinin) or physical pressure (tail compression). In this practical, the students will be able to understand the effects of analgesics on increase in pain threshold and evaluate the antinocecіceptive actions of drugs using hot plate method.

3. Practical outcomes (PrOs):

After completion of this practical, the students will be able to:

PrO	Practical Outcomes	Mapped CO	BTL
PrO1	Explain the mechanism of action of analgesics.	CO2	BTL2
PrO2	Describe the use of hot plate apparatus in evaluation of analgesic drugs.	CO2	BTL2
PrO3	Handle the software for recording the responses.	CO2	BTL3
PrO4	Define algesia and analgesia.	CO2	BTL1

4. Relevant Theoretical Background:

Analgesia

Desensitization to pain without loss of consciousness is called as analgesia. The substance that produces this response is called as analgesics and they produce their action by increasing the threshold for the pain stimuli. The commonly used analgesics are morphine (narcotic analgesics) and aspirin, paracetamol (non-narcotic analgesics).

Pain receptors (Nociceptors), can be excited by three types of stimuli: mechanical, thermal, and chemical. Chemical substances produced by the body that excite pain receptors include bradykinin, serotonin, and histamine. Prostaglandins are fatty acids that are released when inflammation occurs and can increase the pain sensation by sensitizing the nerve endings; that increase in sensitivity is called hyperalgesia.

Opioids are potent pain-relieving medications and are used to treat severe pain. Morphine, an extremely effective analgesic acts by mimicking the endorphins produced naturally by the body by binding to their receptors and blocking or reducing the activation of pain neurons.

The non-narcotic anti-inflammatory analgesics like aspirin, NSAIDs, and COX inhibitors either non selectively or selectively block the activity of COX enzymes. COX enzymes are responsible for the conversion of arachidonic acid (a fatty acid) to prostaglandins, which enhance sensitivity to pain.

Hot plate apparatus

Hot plate apparatus uses heat as source of pain. The plate is heated at constant temperature of 55˚C,on which the individual animal is placed and the reaction of animal, such as hind paw lifting, hind paw licking, or forepaw licking, or jumping response is taken as the end point. The analgesics increase the reaction time. It was proposed by Eddy and Leimbach in 1953.

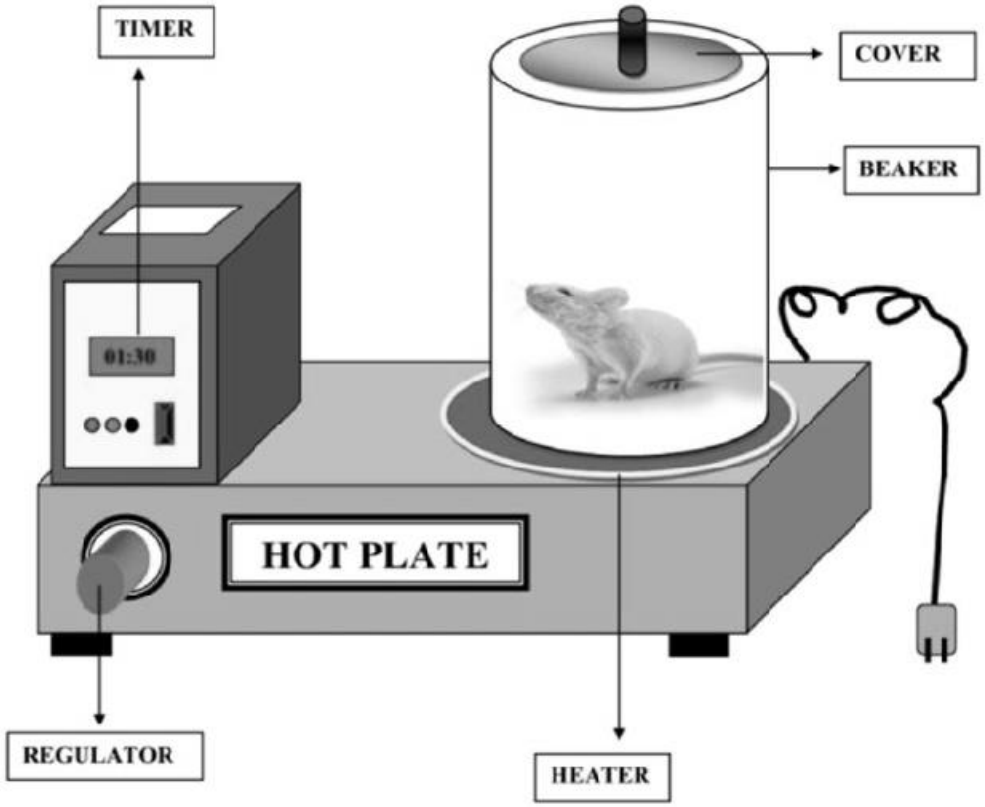


Figure 13.1 Hot Plate Apparatus

5. Precautions:

a. The hot plate method is simple to perform but animals should be accustomed to being handled so that they remain calm during measurements.
b. Do not select the animal if it fails to respond in 6-8 sec (without treatment).
c. The cut off time of 15 seconds is taken as maximum analgesic response to avoid damage to the paw due to heat.

6 Resources required:

Animal: Mice (20-25 g)

Drugs: Morphine sulphate (Dose- 5 mg/kg, S.C., prepare a stock solution containing 0.5 mg/ml and inject1 ml/100 g of body weight of mouse)

Apparatus: Hot plate apparatus

Watch the video of "Study of Analgesic Effect using Hot Plate Method" using the YouTube video or any other video/software/MSBTE CD available.

7. Resources used:

_____

_____

8. Procedure:

a. Weigh and number the animals.
b. Place the individual mouse on the hot plate maintained at constant temperature of 55˚C.
c. Record the basal reaction time by observing the forepaw licking or jump response (whichever appears first) after placing on hot plate. Normally a mouse shows the response within 6-8 sec.
d. Any animal failing to show response in 6-8 sec. should be rejected from the test.
e. Inject the drug morphine s.c. and note the reaction time at a 15, 30, 60, and 120 min after the drug.

f. Cut off time: As the reaction time reaches 15 seconds it is considered maximal analgesia to avoid damage to the paws.

g. Calculate the highest percentage increase in reaction time as index of analgesia at each time interval for individual mouse.

9. Observations:

Record the observation from the software in the following table.

Sr. No.	**Basal reaction time (sec)**	**Reaction time (sec) after drug administration**
	Paw-licking or Jump response	Paw-licking or Jump response
1		
2		
3		
4		
5		
6		
Average		

10. Result:

The reaction time was found to be _____ (increased/decreased).

11. Conclusion:

From the result it can be concluded that the given drugs _____ (possess / does not possess) the analgesic activity.

12. References:

a) Kulkarni SK. Practical Pharmacology and Clinical Pharmacy. First Edition, Vallabh Prakashan, Delhi.

b) MasoumeRezaee-Asl, MandanaSabour, VahidNikoui, SattarOstadhadi, AzamBakhtiarian, "The Study of Analgesic Effects of Leonuruscardiaca L. in Mice by Formalin, Tail Flick and Hot Plate Tests",International Scholarly Research Notices, vol. 2014, Article ID 687697, 5 pages, 2014.

c) mycalpharm.com by infokart India Pvt. Ltd. &XploRemedies LLP.

13. Practical Related Questions (Teacher can give more questions to the student):

a) Explain the mechanism of action of morphine as analgesic in brief.

b) Describe the actions of non-narcotic analgesics.

c) Write the various parts of the hot plate apparatus.

d) Write the pharmacological effects of morphine.

e) What is the dose and route of administration of Morphine sulphate.

(Space for answers)

14. Assessment Scheme

Particular	Understanding the basic concept (Intellectual skill)	Activities (Intellectual and motor skill)	Cleanliness & Handling (Affective domain)	Viva-voce	Total	Signature of teacher
Marks obtained						
Max marks	**2**	**5**	**1**	**2**	**10**	

Experiment No. 14
Study of Analgesic Activity by Writhing Test

1. Aim:

To study of Analgesic Activity by Writhing Test.

2. Practical Significance:

Analgesia is the term used to reduced pain. The Analgesics or antinociceptive agents decrease sensation to pain by increasing the threshold to pain stimuli. A noxious stimulus used to induce painful reaction in animals such as thermal (radiant heat, high temperature), chemicals (Acetic acid, Bradykinin, Prostaglandin) or physical pressure (tail compression). In this practical, the students will be able to understand the effects of certain chemicals that induces painful reactions and the analgesic drugs both narcotic and non –narcotic type that decrease writhing reaction.

3. Practical Outcomes (PrOs):

After completion of this practical, the students will be able to:

PrO	Practical Outcomes	Mapped CO	BTL
PrO1	To identify the writhing response.	CO 2	BTL3
PrO2	Describe the use of writhing method in evaluation of analgesic drugs.	CO2	BTL2
PrO3	Evaluate the antinociceptive effect of drugs, using writhing test.	CO 2	BTL5
PrO3	Define algesia and analgesia.	CO2	BTL1

4. Relevant Theoretical Background:

Analgesia

Analgesia is the loss of sensation of pain without affecting the consciousness. The substance which produces this action is called Analgesics. They produce their action by increasing the threshold for the pain stimuli. The commonly used analgesics are Morphine (Narcotic analgesics) and Aspirin, Paracetamol (Non-narcotic analgesics)

Principle of Writhing test

Writhing test is a chemical method used to induce pain of peripheral origin by intraperitoneal injection of irritant chemicals like phenyl quinone, bradykinin or acetic acid, produces pain reaction which is characterized as a writhing response .Abdominal constriction, turning of trunk (twist) & extension of hind legs (stretching) response by the animal are taken as reaction to chemically induced pain. Analgesics, narcotic and non- narcotic type inhibit writhing response.

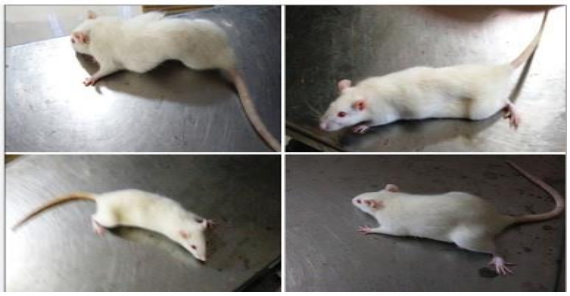


Fig . 14.1 Acetic acid induced writhing response in mice

5. Requirement:

Animals: Mice (25-30g)

Drugs: Acetic acid 0.1ml of 0.6% solution of acetic acid Intra peritoneal route (inject 1ml/100g of body weight), Diclophenac (Dose- 20mg/kg, i.p. and inject the dose as 1 ml/100 g of body weight)

6. Procedure:

a. Weigh and number the animals (n=6)

b. Divide them in two groups of equal size, label first group as control and second as test group.

c. Administer the 0.1ml of 0.6% solution of acetic acid by Intra peritoneal route to the control group.

d. Place individual animal in glass chamber and observe the writhing response.

e. Note the onset of writhes. Count the number of writhes i.e., abdominal contractions, trunk twist or extension of hind limbs, starting from 5 minutes after the injection of acetic acid up to 20 minutes.

f. In the second group, inject the Diclophenac (20mg/kg, i.p.), 30 min after i.p. administration or 15 min after s.c. administration of the acetic acid appropriately.

g. Note the onset of writhes as indicated above.

h. Calculate the mean writhing scores in control and treated group.

i. Calculate the percentage protection against acetic acid-induced writhing from the given formula:

$$\%\ protection = \frac{Nc - Nt}{Nc} \times 100$$

Where, Nc is number of writhing in control, and Nt is the number of writhing in treated group.

j. Calculate the mean and SD (using Microsoft Excel) of number of writhes for the group of mice.

7. Precautions:

a. The writhing test produces sever pain in mice which raises ethical concern regarding its use.

b. This test was withdrawn from Sept. 2004, soon after implementation of report of Ministry of Environmental and Forest, Animal Welfare Division, Govt. of India.

8. Observations:

Record the observation from the Pharmacology software in the following table.

Mice	**Control Group**(Acetic acid 0.1ml + Saline solution)	**Test Group** (Acetic acid 0.1ml + Diclofenac 20mg/kg I.P)
Mice 1		
Mice 2		
Mice 3		

Mice 4		
Mice 5		
Mice 6		
Mean		
SD		

9. Calculations:

$$\%\ \boldsymbol{protection} = \frac{\boldsymbol{Nc - Nt}}{\boldsymbol{Nc}} \times \mathbf{100}$$

10. Result:

The Diclophenac treated group was found to produce number of writhes as _____ SD less than the control group _____ SD and the % protection against the acetic acid-induced writhes was _____%.

11. Conclusion:

From the result it can concluded that the given drug Diclophenac possesses the _____ activity.

12. References:

a) S.K. Kulkarni Practical Pharmacology and Clinical Pharmacy. Fourth edition, Vallabh Prakashan, Delhi.

b) Dannerman PJ. In : Monitoring of analgesia in analgesia in anesthesia and analgesia in laboratory animals. Kon DK, Sally K, Wixson B, White WJ, John G, editors. USA: Academic Press; 1977. Pp.83-99. Ch. 6.

13. Practical Related Questions (Teacher can give more questions to the student):

a) Define Analgesics give four examples.
b) Classify Analgesic- antipyretics with examples.
c) Classify Narcotic-Analgesic with examples.
d) What is Principle behind Writhing test ?
e) Which are the alternative methods used to study analgesic activity?
f) Explain the mechanism of induction of pain by the acetic acid.
g) Calculate and draw a bar graph of response as mean and SD of both control group and test group.

(Space for Answers)

14. Assessment Scheme:

Particular	Understanding the basic concept (Intellectual skill)	Performance of the experiment (Intellectual and motor skill)	Cleanliness & Handling (Affective domain)	Viva-voce / Answers Written	Total	Signature of teacher
Marks Obtained						
Max Marks	**02**	**05**	**01**	**02**	**10**	

Experiment No. 15
Study of Anti-convulsant by Electro-convulsiometer

1. Aim:
To Study the Anticonvulsant Activity using Electro-Convulsiometer.

2. Practical Significance:
There are different types of epilepsy studied in the animal models, viz., Grandmal , Petitmal or Psychomotor type, can be studied in laboratory animals. The Maximal Electro- Shock (MES)-induced convulsions in animals represent Grandmal type of epilepsy. Similarly, Clonic-type of convulsions similar to Petitmal epilepsy in humans can be induced by a chemical agent like Pentylenetetrazol, Strychnine . Audiogenic seizures are generalized seizures triggered by high-intensity acoustic stimulation. In this practical, the students will be able to identify the phases of epilepsy and evaluate the anticonvulsant actions of drugs.

3. Practical Outcomes (PrOs):
After completion of this practical, the students will be able to:

PrO	Practical Outcomes	Mapped CO	BTL
PrO1	Identify the phases of epilepsy.	CO 2,4	BTL 1
PrO2	Describe the use of Electro-convulsiometer in evaluation of anticonvulsants.	CO2,4	BTL 2
PrO3	Define epilepsy and give its types.	CO2	BTL2

4. Relevant Theoretical Background:
Epilepsy

Epilepsy is a neurological illness characterized by aberrant brain activity that results in seizures or episodes of odd behaviour, sensations, and sometimes loss of consciousness. Seizures refer to an electrical disruption in the brain, whereas convulsion is a general term used to describe uncontrollable muscle contractions. The drugs used in the treatment of convulsions are called as Antiepileptic drugs or Anticonvulsant drugs. Anticonvulsants act by blocking voltage- gated sodium channels (e.g. carbamazepine, phenytoin)

Electroconvulsiometer

Electroconvulsiometer was used to induce maximal electroshock seizures in all subjects. Electrodes were applied to both the ear pinna of animals. An electric shock of 150m Amp and 50 hertz lasting for 0.2 seconds was given to induce seizures.

Figure 15.1: Electo-convulsiometer

5. Principle of Electroconvulsometer:

Electroshock-induced seizures (ES) are one of the most well-studied electrical stimulation models. It involves whole-brain stimulation procedures (e.g., 6 Hz in mice and 50–60 Hz in rats) that can be classified as minimal ES or maximum ES. Minimal ES are a hypothetical model of myoclonic seizures that can be generated using current stimulation through corneal electrodes. There are five different phases of maximal electro-shock induced convulsions such as (i) tonic flexion; (ii) tonic extensor; (iii) clonic convulsions; (iv) stupor; and (v) recovery or death. A substance possesses antiepileptic activity if it decreases or abolishes the extensor phase.

Maximal ES and pentylenetetrazole-induced seizure models have been largely employed for antiepileptic drugs screening. However, antiepileptic drugs that protect against partial and nonconvulsive seizures in epileptic patients failed to do so in the maximal ES and pentylenetetrazole-induced models, respectively. Therefore, a better of models is required during the development of antiepileptic drugs.

6. Requirement:

Animal: Mice (20-25 g)

Drugs: Phenytoin (Dose- 25 mg/kg, i.p., prepare a stock solution containing 5 mg/ml and inject 0.1 ml/100 g of body weight of mouse); saline solution

Equipment: Electro-convulsiometer, corneal electrodes (apply 150 mA current for 0.2 sec), stop watch

7. Procedure:

a. Select 12 animals, weigh and then number these animals.

b. Divide them in two groups of equal size, label as control and treated.

c. Inject the saline solution intraperitoneally at dose of 0.1 ml/100 g of mouse.

d. Wait for 30 min.

e. Hold the animal properly; instil one or two drops of saline solution.

h. Place corneal electrodes on the cornea and apply the prescribed current (50-mA fixed current, a 50-60–Hz pulse frequency, a 0.6-ms pulse width and a 0.2-s stimulus duration).

i. Note different stages of convulsions and time spent by animal in each phase of convulsions i.e., (i) tonic flexion; (ii) tonic extensor; (iii) clonic convulsions; (iv) stupor; and (v) recovery or death.

j. Briefly, following stimulus application an immediate severe tonic seizure with maximal extension of the anterior and posterior legs occurs and the body becomes stiffened; at the end of this tonic phase, which usually lasts for 10-15 s, clonic seizures start. It is characterized by paddling movements of the hind limbs and shaking of the body; 20-30 s later, the animal is usually able to come back to an upright position and start moving around, apparently recovering its normal behaviour.

k. Inject phenytoin intraperitoneally to the treated group.

l. Wait for 30 min and subject the animal to elctroconvulsion as described in step e to h.

m. Calculate the mean and standard error mean (SEM) a group for each phase as given in table.

8. Precautions:

a. During stimulus application, the animal should be restrained only by hand and released at the moment of stimulation to permit observation of the seizure throughout its entire course.

b. If bipolar corneal electrodes are used, a drop of an electrolyte/local anaesthetic should be applied into the eyes before placement of the electrodes (not only to ensure adequate electrode contact and

anaesthesia, but, in mice, also to reduce the incidence of fatalities from maximal electroshock seizures almost to zero).

c. Animals should be allowed to have food and water *ad libitum* because starvation increases the severity of maximal ES, prolonging the tonic extensor component, and significantly reduces the seizure threshold

9. Observations:

Record the observation from the Pharmacology software in the following table.

Sr. No.	Treatment	Time (s) in various phases of convulsions				
		Flexion	*Extensor*	*Clonus*	*Stupor*	*Recovery/death*
1	Saline					
2						
3						
4						
5						
6						
Mean ± SEM						
7	Phenytoin					
8						
9						
10						
11						
12						
Mean ± SEM						

10. Calculations:

11. Result:

The phenytoin treated group was found to decrease the time spent in tonic-extensor phase as _____ SEM _____as compared to the control group _____ SEM _____.

12. Conclusion:

From the result it can be concluded that the given drug_____ possesses the Anticonvulsant activity against maximal electro-shock-induced convulsion in mice.

13. References:

a) Kulkarni SK. Practical Pharmacology and Clinical Pharmacy. First edition, Vallabh Prakashan, Delhi.

b)Castel-Branco MM, AlvesGL, FigueiredoIV, Falcão AC, CaramonaMM. The maximal electroshock seizure (MES) model in the preclinical assessment of potential new antiepileptic drugs. Methods Find Exp Clin Pharmacol. 2009; 31(2):101-6.doi: 10.1358/mf.2009.31.2.1338414.

14. Practical Related Questions (Teacher can give more questions to the student):

a) Define epilepsy and give its types.

b) Mention the drug of choice for each type of epilepsy.

c) Write the mechanism of action of phenytoin and barbiturates.

d) Draw a bar graph of response as mean and SEM of both group control and drug treated.

e) Define antiepileptic drugs and give their classification.

(Space for Answers)

15. Assessment Scheme:

Particular	Understanding the basic concept (Intellectual skill)	Performance of the experiment (Intellectual and motor skill)	Cleanliness & Handling (Affective domain)	Viva-voce / Answers Written	Total	Signature of teacher
Marks Obtained						
Max Marks	**02**	**05**	**01**	**02**	**10**	

Experiment No. 16
Study the Muscle Relaxants by Rota Rod Apparatus

1. Aim:
To study the muscle relaxant property of drug using Rota-rod apparatus

2. Practical Significance:
The Rota-rod test can measure riding time or endurance. It can evaluate the balance, grip strength, and coordination of the animals. Motor coordination has traditionally been assessed in mice by this test, in which the animal is placed on a horizontal rod that rotates about its long axis; the animal must walk forward to remain upright and not fall off. Both set speed and accelerating versions of the Rota-rod are available. The horizontal bar also requires strength for adequate performance, particularly of the forelimbs as the mouse initially grips the bar just with the front paws. In this practical, the students will be able to evaluate the effects of the drugs on the motor coordination, strength and muscle relaxation.

3. Practical Outcomes (PrOs):
After completion of this practical, the students will be able to:

PrO	Practical Outcomes	Mapped CO	BTL
PrO1	Identify the muscle coordination, grip strength and relaxation.	CO 2,4	BTL 2
PrO2	Evaluate the effect of drugs on muscle relaxation or coordination.	CO 2,4	BTL 5
PrO3	Handle the software for recording the responses.	CO2, 4	BTL3

4. Relevant Theoretical Background:

Muscle relaxation

Muscle relaxants are used to relieve muscle spasms which may result from some conditions which affect the nervous system. Conditions which may cause muscle spasms include multiple sclerosis, motor neuron disease and cerebral palsy. Muscle spasms and tightness may also follow long-term injuries to the head or back. Muscle spasm can also occur as part of a more short-term condition or injury, such as low back pain or whiplash. Muscle relaxants help the muscles to relax, which may also reduce pain and discomfort.

5. Principle of Rota-rod apparatus:
There are several commercial versions of this apparatus on the market, but some have disadvantages, such as failing to accelerate at an adequate speed to detect motor in coordination (rather than endurance).

The rota-rod shown in Figure is commonly used for testing. The rod is 3 cm in diameter, supported 30 cm above the base of the apparatus. The surface is knurled in a series of parallel ridges along the longitudinal axis, enabling the mice to grip it effectively. The depth of the ridges is a vital detail; if the mouse cannot get a good grip the test will be very much harder; on the other hand, if the grip is too good the mouse will "cartwheel" around the rod by passively holding on to it. The start speed is adjusted to 4 rpm, the acceleration rate to 20 rpm/min. Maximum speed is 40 rpm. Two flanges prevent the mouse from leaving the rod. Their separation is set at 6 cm (maximum).

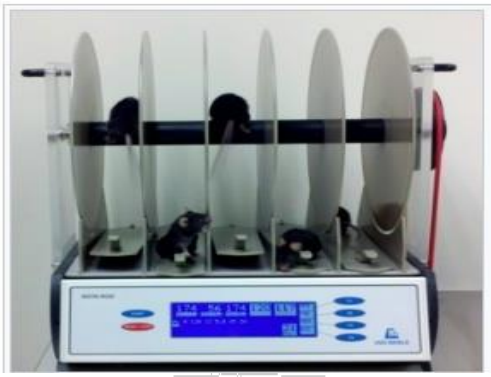


Fig . 16.1 Rota Rod apparatus

6. Requirement:

Animal: Mice (20-25 g)

Drugs: Diazepam (Dose- 4 mg/kg, i.p.); saline solution

Equipment: rota-rod apparatus, stop watch

7. Precautions:

a. Mouse should be practiced first to stay on the rod at the speed of 4 rpm.

b. Mouse should be placed on the moving rod in such a way that it faced towards the rod and moves forward on the rotating rod.

8. Procedure:

Stagnant speed method

a. Select 6 animals, weigh and number them.

b. Turn on the rota-rod apparatus.

c. Maintain the stagnant speed of 20 rpm.

d. Place the animal on the rotating rod facing towards the rod, so that it can move forward.

e. Note down the 'fall off time' when the mouse falls from the rotating rod (normal time 3-5 minutes).

f. After 10 minutes of gap, inject the diazepam to the same animals.

g. Wait for 30 minutes.

h. Place the animal on rotating rod as in step d and note down 'fall off time'.

i. Compare the fall off time before and after the diazepam treatment.

j. Calculate the mean and standard error mean (SEM) for before and after the diazepam treatment as given in table.

9. Observations:

Record the observation from the Pharmacology software in the following table.

Sr. No.	**Treatment and dose**	**Fall off time (s)**		**% decrease in time**
		Before drug	*After drug*	
1.	Diazepam (4 mg/kg)			
2.				
3.				
4.				
5.				
6.				
Mean ± SEM				

10. Result:

The diazepam treatment was found to decrease the fall off time as _____ as compared to before treatment _____.

11. Conclusion:

From the result it can be concluded that the given drug_____ possesses the muscle relaxant activity.

12. References:

a) Robert M.J. Deacon. Measuring Motor Coordination in Mice. J Vis Exp. 2013; (75): 2609.doi: 10.3791/2609

b) Kulkarni SK. Practical Pharmacology and Clinical Pharmacy. First edition, Vallabh Prakashan, Delhi.

13. Practical Related Questions (Teacher can give more questions to the student):

a) Describe the mechanism of action of muscle relaxation.

b) Name the three drugs that produce muscle relaxation and used for muscle spasm.

c) Describe the rota-rod apparatus present in your laboratory.

d) Draw a bar graph of response as mean and SEM before and after drug treated.

e) Write the uses of muscle relaxants.

(Space for Answers)

14. Assessment Scheme:

Particular	Understanding the concept(Intellectual skill)	Observation & Results(Intellectual and motor skill)	Cleanliness(Affective domain)	Viva-voce /Answers Written	Total	Signature of teacher
Marks Obtained						
Max Marks	**02**	**05**	**01**	**02**	**10**	

Experiment No. 17
Study of CNS stimulants by Actophotometer

1. Aim:
To study the CNS stimulants using Actophotometer

2. Practical Significance:
Central nervous system stimulants cause release of certain neurotransmitters in brain or inhibit inhibitory neurotransmitters activity to induce stimulation. Most of the CNS stimulants increase the locomotor activity in animals and humans. The stimulants like caffeine and amphetamine increases locomotor activity. Examination of locomotor activity gives the indications for wakefulness of brain. In this practical, the student will be able to evaluate the effects of the drugs on the locomotor activity using an Actophotometer.

3. Practical Outcomes (PrOs):
After completion of this practical, the students will be able to:

PrO	Practical Outcomes	Mapped CO	BTL
PrO1	Identify the effects of drugs on locomotor activity.	CO 2,4	BTL 2
PrO2	Evaluate the effect of drugs on CNS.	CO2,4	BTL5
PrO3	Operate the actophotometer apparatus efficiently	CO2, 4	BTL3

4. Relevant Theoretical Background:
Actophotometer apparatus Instrumentation

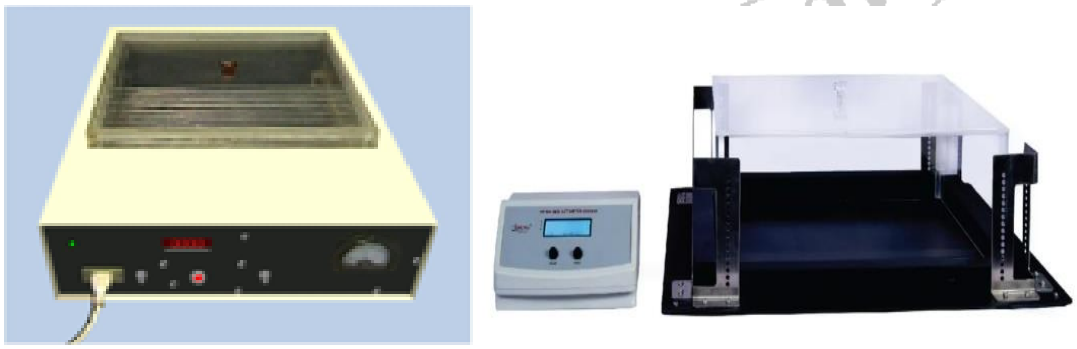
An Actophotometer consist of :

a. Computer screen with Orchid Scientific Software Some of the actophotometer are also available along with recording devices. In this software catches the motion of animals and record the locomotor activity.

b. Activity area with Photoelectric cell: An actophotometer measures the locomotor activity. When the beam of light falling on the photoelectric cell cut off due to locomotion of animals, a count is recorded. The number of counts in certain time by animals indicates locomotor activity.

c. Digital Counter: Which is attached to activity area, which shows fluctuations in digital counter screen due to movement of rat to measure locomotor activity?

We can use both mice and rats for screening locomotor activity.



(A: Old Version) **(B: Latest Version)**

Fig .17.1 Actophotometer

5. Requirement:

Animal: Rat (100-250 g)

Drugs: Caffeine, Dose- 3 mg/kg,i.p, saline solution

Equipment: Orchids Scientific Actophotometer apparatus, Computer screen and CPU

6. Precautions:

a. Cleaning of Actophotometer after each trial on animal is required to removes the physical cues of animals, as it may interfere in the results.

7. Procedure:

a. Select 6 healthy rat of either sex, weigh them and number them.

b. Turn on the Actophotometer apparatus.

c. Check whether the entire photoelectric cell are working well and counting the cut offs by interrupting the beams with hand movement.

d. Place individually each Rat in the activity area.

e. Record and note down the basal activity of the Rat for 5 minutes.

f. Inject the caffeine and after 30 min, re-test the individual Rat for locomotor activity for 5 minutes.

g. Compare the locomotor counts before and after drug treatment.

h. Repeat the same procedure for remaining 5 rats.

i. Calculate the mean and standard error mean (SEM) for before and after the drug treatment as given in table.

8. Observations:

Record the observation from the Pharmacology software in the following table.

Sr. No.	**Treatment and dose**	**Locomotor counts**		**% increase**
		Before drug	*After drug*	
1.	Caffeine, 3mg/kg, i.p			
2.				
3.				
4.				
5.				
6.				
Mean ± SEM				

9. Result:

The _____ treatment was found to increase the locomotor as _____ as compared to before treatment _____.

10. Conclusion:

From the result it can be concluded that the given drug_____ possesses the CNS stimulant activity.

11. References:

a) Kulkarni SK. Practical Pharmacology and Clinical Pharmacy. First edition, Vallabh Prakashan, Delhi.

12. Practical Related Questions (Teacher can give more questions to the student):

a) Can we use this apparatus to verify antianxiety or anti-schizophrenic activity of drugs, why?

b) Name the some Stimulatory neurotransmitters in the brain.

c) Explain the mechanism of action of amphetamine or caffeine.

d) Draw a bar graph of response as mean and SEM before and after drug treated.

e) What type of activity drug has on CNS? Justify answer based on observation.

f) What is the dose and route of administration of Caffeine?

(Space for Answers)

13. Assessment Scheme:

Particular	Understanding the basic concept (Intellectual skill)	Performance of the experiment (Intellectual and motor skill)	Cleanliness & Handling (Affective domain)	Viva-voce /Answers Written	Total	Signature of teacher
Marks Obtained						
Max Marks	**02**	**05**	**01**	**02**	**10**	

Experiment No.18
Study of CNS depressants by Actophotometer

1. Aim:

To study the CNS depressants using Actophotometer

2. Practical Significance:

Central nervous system depressants causes release of certain neurotransmitter in brain or inhibit stimulatory neurotransmitters activity to induce sedation. Most of the CNS depressants decrease the locomotor activity in animals and humans. The depressants like diazepam, alcohol, barbiturates decreases locomotor activity. Examination of locomotor activity gives the indications for wakefulness of brain. In this practical, the student will be able to evaluate the effects of the drugs on the locomotor activity using an Actophotometer.

3. Practical Outcomes (PrOs):

After completion of this practical, the students will be able to:

PrO	Practical Outcomes	Mapped CO	BTL
PrO1	Identify the effects of drugs on locomotor activity.	CO 2,4	BTL 2
PrO2	Evaluate the effect of drugs on CNS.	CO 2,4	BTL5
PrO3	Operate the actophotometer apparatus efficiently	CO2, 4	BTL3

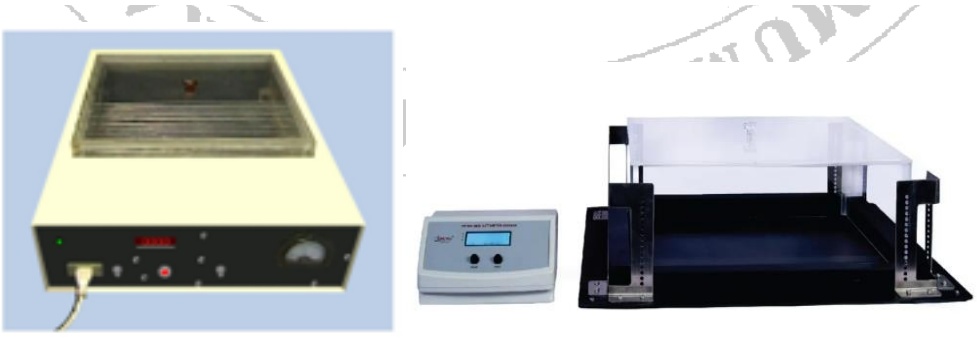
4. Relevant Theoretical Background :

Actophotometer apparatus

An Actophotometer measures the locomotor activity (horizontal activity). It consists of photoelectric cell and the digital counter on opposite side. When the beam of light falling on the photoelectric cell cut off due to locomotion of animals, a count is recorded. The number of counts in certain time by animals indicates locomotor activity. An actophotometer apparatus is available in circular or square areas in which the animal moves.

Some of the Actophotometer are also available along with recording devices. In this software catches the motion of animals and record the locomotor activity.

Apparatus is available for both mice and rats.



(A: Old Version) **(B: Latest Version)**

Fig . 18.1Actophotometer

5. Requirement:

Animal: Rat (100-250 g)

Drugs: Diazepam Dose- 5 mg/kg, i.p., saline solution

Equipment: Orchids Scientific Actophotometer apparatus, Computer screen and CPU

6. Precautions:

Cleaning of Actophotometer after each trial on animal is required to remove the physical cues of animals, as it may interfere in the results.

7. Procedure:

a. Select 6 healthy rat of either sex , weigh them and number them.

b. Turn on the Actophotometer apparatus.

c. Check whether the entire photoelectric cell are working well and counting the cut offs by interrupting the beams with hand movement.

d. Place individually each Rat in the activity area.

e. Record and note down the basal activity of the Rat for 5 minutes.

f. Inject the Diazepam and after 30 min, re-test the individual Rat for locomotor activity for 5 minutes.

g. Compare the locomotor counts before and after drug treatment.

h. Repeat the same procedure for remaining 5 rats.

i. Calculate the mean and standard error mean (SEM) for before and after the drug treatment as given in table.

8. Observations:

Record the observation from the Pharmacology software in the following table.

Sr. No.	**Treatment and dose**	**Locomotor counts**		**% Decreases**
		Before drug	*After drug*	
1.	Diazepam 5mg/kg i.p			
2.				
3.				
4.				
5.				
6.				
Mean ± SEM				

9. Result:

The _____ treatment was found to decrease the locomotor as _____ as compared to before treatment_____.

10. Conclusion:

From the result it was concluded that the given drug_____ possesses the CNS depressant activity.

11. References:

a) Kulkarni SK. Practical Pharmacology and Clinical Pharmacy. First edition, Vallabh Prakashan, Delhi.

12. Practical Related Questions (Teacher can give more question to the students):

a) Why chlorpromazine decreases locomotor activity?

b) Name the some inhibitory neurotransmitters in the brain.

c) Explain the mechanism of action of barbiturates.

d) Draw a bar graph of response as mean and SEM before and after drug treated.

e) What is the dose and route of administration of Diazepam?

(Space for Answers)

13. Assessment Scheme:

Particular	Understanding the basic concept (Intellectual skill)	Performance of the experiment (Intellectual and motor skill)	Cleanliness & Handling (Affective domain)	Viva-voce /Answers Written	Total	Signature of teacher
Marks Obtained						
Max Marks	**02**	**05**	**01**	**02**	**10**	

Experiment No. 19
Study the Anxiolytic Activity by Elevated plus Maze Apparatus

1. Aim:

To study the Anxiolytic activity of drug using elevated plus maze apparatus

2. Practical Significance:

Any fearful or conflict condition produces anxiety in humans as well as in animals. Anxiolytic or anti-anxiety agents can decrease the effect of conflict and increases the performance. Elevated plus maze (EPM) apparatus induces approach avoidance conflict. In this practical, the students will be able to identify the natural behaviour of animal and evaluate the anxiolytic activity of drugs using elevated plus maze apparatus.

3. Practical Outcomes (PrOs):

After completion of this practical, the students will be able to:

PrO	Practical Outcomes	Mapped CO	BTL
PrO1	Identify the natural response of animal in elevated plus maze.	CO 2,4	BTL 2
PrO2	Evaluate the effect of drugs on central nervous system.	CO 2,4	BTL5
PrO3	Describe the use of the elevated plus maze in evaluation of antianxiety drugs.	CO2, 4	BTL2

4. Relevant Theoretical Background:

Anxiety

Anxiety is a body's natural response to stress. It is a feeling of fear, dread, and uneasiness. Anxiolytic agents relieve the symptoms of the anxiety.

Elevated plus maze

Elevated plus maze test has predictive validity that all the antianxiety agents show an effect in the test. It has two counter open arms and enclosed arms. It has two open arms (16 × 5 cm) and two enclosed arms (16 × 5 × 12 cm) with an open roof. The plus maze is elevated at height of 25 cm. Animals expose to a novel environment that is situated at height evokes an approach-avoidance conflict which is stronger in open arm as compared to enclosed arm. Animals show fear like responses and spend more time in enclosed arm, when in open arm it freeze, defecate and show fear-like movements. Moreover, blood cortisol level (parameter of aversion and fear) also increases that is a true reflection of anxiety. Elevated plus maze is simple, fast and less time consuming test to evaluate Antianxiety agents. There is no prior training required to the animals. Apparatus is available for both mice and rats.

Figure: Elevated Plus Maze

5. Precaution:

a. Cleaning of elevated plus maze after each test on animal is required to removes the physical cues of animals, as it may interfere in the results.

6. Requirements:

Animal: Mice (20-25 g)

Drugs: Diazepam (Dose- 2 mg/kg, i.p); and inject 1 ml/100 g of body weight of mouse; saline solution

Equipment: Elevated plus maze apparatus, stop watch

7. Procedure:

a. Select 10-12 animals, weigh, divide in equal group and number them.

b. Take an animal from control group (saline treated group), place them individually in the centre of the maze, head facing towards open arm and start the stop watch.

c. Note down the following parameters for 5 minutes:

i. First preference of mouse to open or enclosed arm.

ii. Number of entries in open and enclosed arm (an arm entry should note only when mouse enters in arm with four paws)

iii. Average time spent by each animal in each arm (average time = total duration in the arm/number of entries)

d. Inject the drug and after 30 min, place the individual mouse in the centre of the arm, head facing towards open arm.

e. Note the parameters as given above in step c for 5 minutes.

f. Compare the preference of the animal to open/enclosed arm, average time spent in open arm and number of entries in open arm in each group.

g. Calculate the percent preference for a group.

h. Calculate the mean and standard error mean (SEM) for before and after the drug treatment as given in table.

8. Observations:

Record the observation from the Pharmacology software in the following table.

Sr. No.	**Treatment and dose**	**Preference to open arm (Y or N)**	**Open arm**	
			Number of entries	Average time spent (s)
1.	Normal saline solution			
2.				
3.				
4.				
5.				
6.				
		(% preference to open arm)	(Mean ± SEM)	(Mean ± SEM)

Sr. No.	Treatment and dose	Preference to open arm (Y or N)	Open arm	
			Number of entries	Average time spent (s)
1.	Diazepam 2 mg/kg, i.p			
2.				
3.				
4.				
5.				
6.				
		(% preference to open arm)	(Mean ± SEM)	(Mean ± SEM)

9. Result:

The _____ treatment was found to increase the % preference and average time spent in open arm as _____ SEM _____, _____ SEM _____ respectively as compared to control group _____ SEM _____, _____ SEM _____.

10. Conclusion:

From the result it was concluded that the given drug_____ possesses the anxiolytic activity.

11. References:

a) Kulkarni SK. Practical Pharmacology and Clinical Pharmacy. I edition, Vallabh Prakashan, Delhi.

12. Practical Related Questions (Teacher can give more questions to the student):

a) Explain the role sympathetic system in anxiety.
b) Describe the mechanism of Benzodiazepines.
c) Give the dimensions of the elevated plus maze test apparatus used for the rats.
d) Write principle behind elevated plus maze test apparatus.
e) Draw a bar graph of response as mean and SEM before and after drug treated.

(Space for answers)

13. Assessment Scheme:

Particular	Understanding the basic concept (Intellectual skill)	Performance of the experiment (Intellectual and motor skill)	Cleanliness & Handling (Affective domain)	Viva-voce /Answers Written	Total	Signature of teacher
Marks Obtained						
Max Marks	**02**	**05**	**01**	**02**	**10**	

Experiment No. 20

Study of effect of Sympathomimetics (Adrenaline) on isolated heart

1. Aim:

To study the effect of adrenaline on isolated frog heart.

2. Practical Significance:

Adrenaline is a hormone produced within the adrenal gland, in response to stress, it increases heart rate, strengthens the force of the heart's contraction and cardiac output, and increases blood pressure. Adrenaline is a used to treat the symptoms of cardiac arrest, hypotension associated with septic shock, severe allergic reaction (anaphylaxis), symptomatic bradycardia. In this practical, the students will be able to identify and evaluate the effects of adrenaline on isolated frog heart.

3. Practical Outcomes (PrOs):

After completion of this practical, the students will be able to:

PrO	Practical Outcomes	Mapped CO	BTL
PrO1	Identify and observe the heart rate.	CO 2,3,4	BTL 2
PrO2	Identify and observe the force of contraction.	CO 2,3,4	BTL 2
PrO3	Identify and observe the tone.	CO 2,3,4	BTL 2
PrO4	Handle the software for recording the responses.	CO2, 3, 4	BTL3

4. Relevant Theoretical Background:

Sympathomimetic drugs

Sympathomimetic drugs mimic the responses obtained by the stimulation of sympathetic or adrenergic nerves. Ex. Adrenaline, Noradrenaline, Dobutamine, Isoprenaline, Dopamine.

Drugs may influence the rate and force of contraction of the heart. An increase in the heart rate is called positive chronotropic effect, while decrease in heart rate is negative chronotropic effect. Similarly increase in force of contraction is called a positive inotropic effect and a decrease in force of contraction is called a negative inotropic effect.

Adrenaline is a hormone derived from tyrosine—an amino acid. Adrenaline is produced by the chromaffin cells in the medulla of the adrenal glands and is released in response to a stressor or perceived threat. This stressor can be emotional, physical or environmental. Adrenalin stimulates the liver to break down glycogen into glucose (to provide quick energy to the body), relax the smooth muscles in the lungs and respiratory tract to enhance inspiration and lung capacity, Stimulates the beta-adrenergic receptors in the myocardium to increase cardiac contractility and heart rate, contracts the arteries in the skin to divert blood flow and contract smooth muscles in the skin, causing the hairs to raise on the skin surface (goose bumps).

Sympathomimetic amines such as adrenalin and Nor-adrenalin produce positive chronotropic and positive inotropic effect by acting on adrenergic beta one receptors which are present in heart where as parasympathomimetic drug such as acetylcholine produce negative chronotropic and negative inotropic effect by acting on muscarinic receptors in heart.

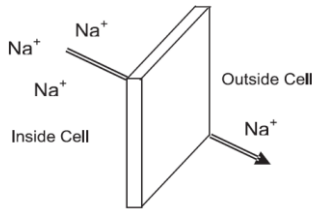
Action potential

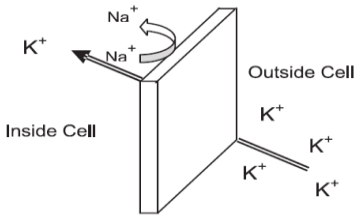
A phenomenon of continuously varying potential difference across the cell membrane is called as action potential. The cardiac cells have an unusually long action potential, which can be divided into five phases (0-4).

Phase 0: Depolarization

The depolarization occurs when the cell membrane reaches the threshold voltage and

Na ion channels opens resulting in fast inward current.





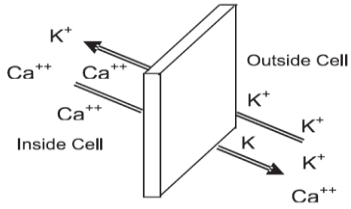
Phase 0: Depolarization **Phase 1: Partial repolarization**

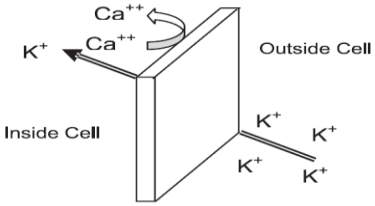
Phase 1: Partial repolarization

In phase 1, the inactivation of Na^+ ion channels takes place while K^+ ion channels are rapidly opened and closed causing transient outward current.

Phase 2: Plateau

In phase 2, Ca^{2+} ion channels opened resulting in slow inward current (It balances the slow outward leak K^+ ions)





Phase 2: Plateau Phase **Phase 3: Repolarization**

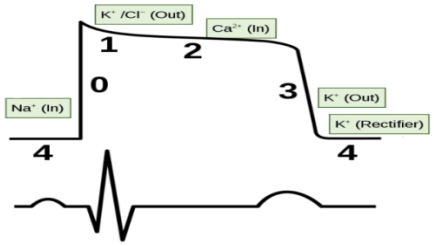
Phase 3: Repolarization

In this phase, Ca^{2+} ion channels closed and K^+ ion channels opened resulting in outward current.

Phase 4. Forward current

In this phase, Increase in depolarization takes place due to increase in Na^+ permeability.

Spontaneous depolarization brings the cells to threshold of next action potential.



Physiological salt solution

The physiological salt solutions are used to keep isolated tissue and organ preparations surviving throughout the experiment. Frog ringer is the physiological solution of choice for frogs heart preparation.

Composition of frog ringer solution

Sr. No	Composition	Function
1	Sodium chloride	To provide isotonicity, contractility, excitability
2	Potassium chloride	To provide ionic balance
3	Calcium chloride	To provide contractility
4	Sodium Bicarbonate	To provide alkaline medium
5	Sodium Dihydrogen phosphate	As a buffer
6	Glucose	To provide energy
7	water	---

5. Precautions:

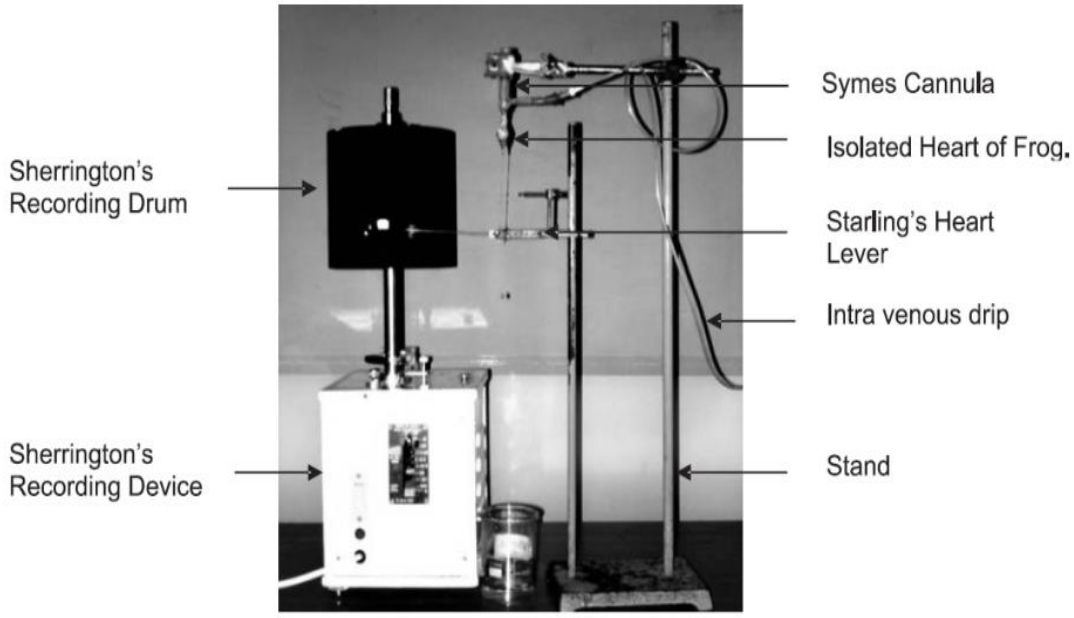
1. Remove the blood from the heart completely after giving the small cut (blood causes formation of clot and heart stops.)
2. Give sufficient time for the heart to recover between the two doses of drug by taking the baseline every time.

6. Requirements:

Computer and any software of animal experimentation (For actual experimentation: Adrenalin 100 microgram/ml, Frog ringer solution, frog, kymograph paper, starling heart lever, L-stand T-rod, X-blocks, Syme's cannula, screw clip, marriotte bottle, rubber tubes, tuberculine syringe, 26 no. needle and surgical instrument box).

7. Procedure:

a. Set up the assembly for experiment.
b. Pith the frog by passing the needle through the occipito-atlantic junction between the brain and spinal cord. The stretching out of limbs indicates that the pithing is proper.
c. Place the frog in a tray with ventral side facing up.
d. Make an incision on the skin longitudinally and the expose the rectus abdominal muscle.
e. Make an incision around the rectus muscle without damaging the anterior abdominal vein.
f. Expose the heart after cutting the sternum.
g. Carefully remove the pericardium and put a few drops of frog ginger over the heart.
h. Trace the inferior Vena Cava, pass a thread below it and give a small cut in order insert the venous cannula, which in turn connected to a perfusion bottle containing frog's ringer.
i. Insert the cannula in the vena cava and tie the thread to assure the cannula in place.
j. Give small cut in one of the aorta for the perfusate to come out.
k. Adjust a proper venous pressure by using marriott's bottle, which helps in attaining the constant pressure. Start the perfusion by opening screw clamp attached to the tube.
l. Cut the other parts attached to the heart and isolate the heart.
m. Mount the heart by fixing the cannula in screw clamp.
n. Attach one end of thread to the lower part of the ventricle and other end of thread to the Starling's heart liver.
o. Adjust the tip of starling heart liver on the drum fixed to Sherrington's rotating machine for recording the response.
p. Record the normal heart beat on the paper of the drum.
q. Inject the graded doses of 0.1 ml of adrenaline solution into Syme's cannula.
r. Immediately switch on the kymograph and record the effect of adrenaline for 2 minutes period. After 2 minutes switch off the kymograph till the heat beat and amplitude comes to the normal.
s. Again record normal response of heart and inject 0.2 ml of adrenalin and repeat step 18.
t. Similarly give injection of 0.4 ml and 0.8 ml and repeat the steps and record the responses.
u. After completion of doses, label and fix the graph with fixing solution.
v. Observe the response of adrenaline and record in observation table.



Assembly for isolated heart of a frog experiment

Graphical Record

8. Observations:

Record the observation from the Pharmacology software in the following table. Observe and record heart rate, force of contraction and tone.

Sr. No.	Dose of adrenalin (ml)	Heart rate (Beats/min)	Force of contraction	Tone
1	Frogs ringer	30-50	Normal	Normal
2	0.1 ml			
3	0.2 ml			
4	0.4 ml			
5	0.8 ml			

9. Result:

1. Adrenalin was found to increase / decrease heart rate
2. Adrenalin was found to increase / decrease force of contraction.
3. Adrenalin was found to increase / decrease tone.

10. Conclusion:

From the result it can be concluded that adrenalin shows …….. inotropic / chronotropic effect on heart.

11. References:

a) Kulkarni SK. Practical Pharmacology and Clinical Pharmacy. 1st edition, Vallabh Prakashan, Delhi.

b) Badyal D. Practical manual of pharmacology. 1st edition, Jaypee brothers medical publishers, New Delhi.

12. Practical Related Questions (Teacher can give more questions to the student):

a) Which type of effect is produced by adrenalin?

b) Name adrenergic receptor present on heart.

c) Why adrenalin increases heart rate and force of contraction?

d) Draw the graph of effect of adrenalin on isolated heart of a frog.

e) Name the parts of isolated heart of a frogs assembly.

f) List five drugs shows sympathomimetic action.

(Space for answers)

13. Assessment scheme:

Particular	Understanding the basic concept (Intellectual skill)	Performance of the experiment (Intellectual and motor skill)	Cleanliness & Handling (Affective domain)	Viva-voce / Answers Written	Total	Signature of teacher
Marks Obtained						
Max Marks	**02**	**05**	**01**	**02**	**10**	

Experiment No. 21

Study of effect of Parasympathomimetic (Acetylcholine) on isolated heart

1. Aim:

To study the effect of Acetylcholine on isolated frog heart.

2. Practical Significance:

Acetylcholine (ACh) is a Parasympathomimetic drug which mimics the effect of parasympathetic nerve stimulation. ACh is released as a neurotransmitter from the vagus nerve (Cranial Nerve X,) resulting in a decrease in the cardiac rate. Acetylcholine acts on muscarinic receptors as an agonist and decreases the heart rate and amplitude. In cardiovascular system ACh acts as a vasodilator, decreases heart rate, and decreases heart muscle contraction. It helps to regulate heartbeat, blood pressure, and heart muscle contractions. In this practical students will able to identify and evaluate the effects of ACh on isolated frog heart.

3. Practical Outcomes (PrOs):

After completion of this practical, the students will be able to:

PrO	Practical Outcomes	Mapped CO	BTL
PrO1	Identify and observe the heart rate.	CO 2,3,4	BTL 2
PrO2	Identify and observe the force of contraction.	CO 2,3,4	BTL 2
PrO3	Identify and observe the tone.	CO 2,3,4	BTL 2
PrO4	Handle the software for recording the responses.	CO2, 3,4	BTL3

4. Relevant Theoretical Background:

Drugs may influence the rate and force of contraction of the heart. An increase in the heart rate is called positive chronotropic effect, while decrease in heart rate is negative chronotropic effect. Similarly increase in force of contraction is called a positive inotropic effect and a decrease in force of contraction is called a negative inotropic effect.

Acetylcholine, an ester of choline and acetic acid that serves as a transmitter substance of nerve impulses within the central and peripheral nervous systems. Acetylcholine is the chief neurotransmitter of the parasympathetic nervous system, the part of the autonomic nervous system (a branch of the peripheral nervous system) that contracts smooth muscles, dilates blood vessels, increases bodily secretions, and slows heart rate. Acetylcholine can stimulate a response or block a response and thus can have excitatory or inhibitory effects.

Acetylcholine is stored in vesicles at the ends of cholinergic (acetylcholine-producing) neurons. In the peripheral nervous system, when a nerve impulse arrives at the terminal of a motor neuron, acetylcholine is released into the neuromuscular junction. There it combines with a receptor molecule in the postsynaptic membrane (or end-plate membrane) of a muscle fiber. This bonding changes the permeability of the membrane; causing channels to open that allow positively charged sodium ions to flow into the muscle cell (see end-plate potential). If successive nerve impulses accumulate at a sufficiently high frequency, sodium channels along the end-plate membrane become fully activated, resulting in muscle cell contraction.

Within the autonomic nervous system, ACh being discharged from the terminal of one neuron and binding to receptors on the postsynaptic membrane of other cells. Its activities within the autonomic nervous system affect a number of body systems, including the cardiovascular system, where it acts

as a vasodilator, decreases heart rate, and decreases heart muscle contraction. It acts on muscarinic M2 receptor and produces negative chronotropic and negative ionotropic effect on heart.

Acetylcholine is rapidly destroyed by the enzyme acetylcholinesterase. Inhibitors of the enzyme (drugs known as anticholinesterases) prolong the lifetime of acetylcholine such as Physostigmine and Neostigmine.

5. Precautions:

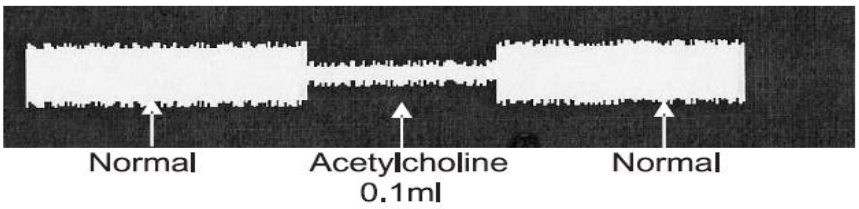
1. Remove the blood from the heart completely after giving the small cut (blood causes formation of clot and heart stops.)
2. Give sufficient time for the heart to recover between the two doses of drug by taking the baseline every time.

6. Requirements:

Computer and any software of animal experimentation (For actual experimentation: Acetylcholine 100 microgram/ml, frog ringer solution, frog, kymograph paper, starling heart lever, L-stand T-rod, X-blocks, Syme's cannula, screw clip, mariotte bottle, rubber tubes, tuberculine syringe, 26 no. needle and surgical instrument box).

7. Procedure:

a. Set up the assembly for experiment.
b. Pith the frog by passing the needle through the occipito-atlantic junction between the brain and spinal cord. The stretching out of limbs indicates that the pithing is proper.
c. Place the frog in a tray with ventral side facing up.
d. Make an incision on the skin longitudinally and the expose the rectus abdominal muscle.
e. Make an incision around the rectus muscle without damaging the anterior abdominal vein.
f. Expose the heart after cutting the sternum.
g. Carefully remove the pericardium and put a few drops of frog ginger over the heart.
h. Trace the inferior Vena Cava, pass a thread below it and give a small cut in order insert the venous cannula, which in turn connected to a perfusion bottle containing frog's ringer.
i. Insert the cannula in the vena cava and tie the thread to assure the cannula in place.
j. Give small cut in one of the aorta for the perfusate to come out.
k. Adjust a proper venous pressure by using marriott's bottle, which helps in attaining the constant pressure. Start the perfusion by opening screw clamp attached to the tube.
l. Cut the other parts attached to the heart and isolate the heart.
m. Mount the heart by fixing the cannula in screw clamp.
n. Attach one end of thread to the lower part of the ventricle and other end of thread to the Starling's heart liver.
o. Adjust the tip of starling heart liver on the drum fixed to Sherrington's rotating machine for recording the response.
p. Record the normal heart beat on the paper of the drum.
q. Inject the doses of 0.1 ml of ACh (10 mcg/ml) solution into Syme's cannula.
r. Immediately switch on the kymograph and record the effect of ACh for 2 minutes period. After 2 minutes switch off the kymograph till the heat beat and amplitude comes to the normal.



s. Again record normal response of heart and inject 0.2 ml of ACh and repeat step 18.

t. Similarly give injection of 0.4 ml and 0.8 ml and repeat the steps and record the responses.

u. After completion of doses, label and fix the graph with fixing solution.

v. Observe the response of ACh and record in observation table.

8. Observations:

Record the observation from the Pharmacology software in the following table. Observe and record heart rate, force of contraction and tone.

Sr. No.	Dose of ACh (ml)	Heart rate (Beats/min)	Force of contraction	Tone
1	Frogs ringer	30-50	Normal	Normal
2	0.1 ml			
3	0.2 ml			
4	0.4 ml			
5	0.8 ml			

9. Result:

1. ACh was found to increase / decrease heart rate.
2. ACh was found to increase / decrease force of contraction.
3. ACh was found to increase / decrease tone.

10. Conclusion:

From the result it can be concluded that ACh shows …….. inotropic and chronotropic effect on heart.

11. References :

a) Kulkarni SK. Practical Pharmacology and Clinical Pharmacy. 1st edition, Vallabh Prakashan, Delhi.

b) Badyal D. Practical manual of pharmacology. 1st edition, Jaypee brother's medical publishers, New Delhi.

12. Practical Related Questions (Teacher can give more questions to the student):

a) What is the effect of ACh on heart?

b) Name ACh receptor present on heart.

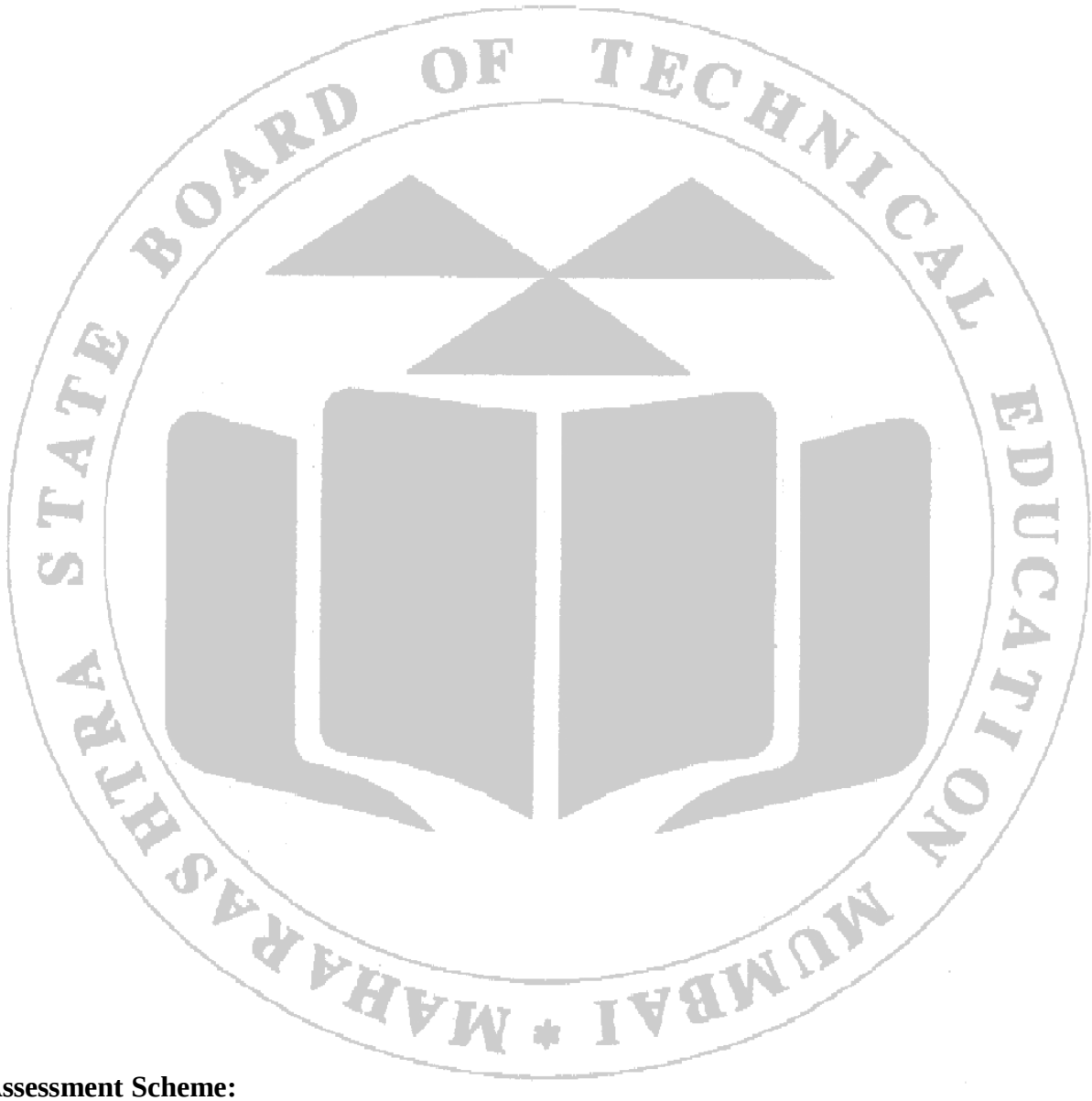
c) Why ACh decreases heart rate and force of contraction?

d) Draw the graph of effect of ACh on isolated heart of a frog.

e) List five drugs shows parasympathomimetic action.

f) Which enzyme destroys ACh?

(Space for answers)



13. Assessment Scheme:

Particular	Understanding the basic concept (Intellectual skill)	Performance of the experiment (Intellectual and motor skill)	Cleanliness & Handling (Affective domain)	Viva-voce / Answers Written	Total	Signature of teacher
Marks Obtained						
Max Marks	**02**	**05**	**01**	**02**	**10**	

Experiment No.22

Study of effect of Calcium and Potassium ions on isolated heart

1. Aim:

To study the effect of calcium and potassium ions on isolated heart.

2. Practical Significance:

The heart is a pump that brings blood to every part of the body. Calcium plays important roles in the electrical activity and pumping function of the heart. Calcium enters the heart muscle cells during each heart beat and contributes to the electrical signal that coordinates the heart's function. Calcium also binds to machinery within the cell that helps the cell to squeeze together ("contract"), which makes the heart pump blood. Low levels of calcium has been linked with heart failure, low blood pressure (hypotension) and life threatening rhythm disorders of the heart whereas higher calcium levels increases risk of developing coronary artery disease and heart attack.

Potassium plays an important role in regulating the contractions of all muscles, including the heart muscle. Very low levels of potassium in the body can lead to irregular heart rhythms, including sinus bradycardia, ventricular tachycardia, and ventricular fibrillation while too much potassium, heart may beat irregularly, which in the worst cases can cause heart attack.

In this practical students will able to identify and evaluate the effects of calcium and potassium ions on isolated frog heart.

3. Practical Outcomes (PrOs):

After completion of this practical, the students will be able to:

PrO	Practical Outcomes	Mapped CO	BTL
PrO1	Identify and observe the heart rate.	CO 2,3,4	BTL 2
PrO2	Identify and observe the force of contraction.	CO 2,3,4	BTL 2
PrO3	Identify and observe the tone.	CO 2,3,4	BTL 2
PrO4	Handle the software for recording the responses.	CO2, 3,4	BTL3

4. Relevant Theoretical Background:

Calcium ions are essential for vigorous contraction of the heart muscle. If calcium concentration is increased, the heart contract vigorously and fails to relax completely during successive beats and finally stops in systole (contraction). Calcium which enters in the cells causes release of calcium stored in the sarcoplasmic reticulum by acting on ryanodine receptors which raises the concentration of calcium within the cells. This intracellular free calcium then interacts with troponin-actin-myosin system and causes contraction of the heart muscle. Potassium ions are present intracellularly which are responsible for relaxation of heart muscle. If potassium ion concentration is increased it causes heart to relax completely and stops in the diastole (relaxation).

5. Precautions:

a. Remove the blood from the heart completely after giving the small cut (blood causes formation of clot and heart stops.)

b. Give sufficient time for the heart to recover between the two doses of drug by taking the baseline every time.

6. Requirements:

Computer and any software of animal experimentation (For actual experimentation: Calcium chloride (4%), Potassium chloride (4%) frog ringer solution, frog, kymograph, sterling heart lever, L-stand T-rod, X-blocks, Syme's cannula, screw clip, mariotte bottle, rubber tubes, tuberculine syringe, 26 no. needle and surgical instrument box).

7. Procedure:

a. Set up the assembly for experiment.

b. Pith the frog by passing the needle through the occipito-atlantic junction between the brain and spinal cord. The stretching out of limbs indicates that the pithing is proper.

c. Place the frog in a tray with ventral side facing up.

d. Make an incision on the skin longitudinally and the expose the rectus abdominal muscle.

e. Make an incision around the rectus muscle without damaging the anterior abdominal vein.

f. Expose the heart after cutting the sternum.

g. Carefully remove the pericardium and put a few drops of frog ginger over the heart.

h. Trace the inferior Vena Cava, pass a thread below it and give a small cut in order insert the venous cannula, which in turn connected to a perfusion bottle containing frog's ringer.

i. Insert the cannula in the vena cava and tie the thread to assure the cannula in place.

j. Give small cut in one of the aorta for the perfusate to come out.

k. Adjust a proper venous pressure by using marriott's bottle, which helps in attaining the constant pressure. Start the perfusion by opening screw clamp attached to the tube.

l. Cut the other parts attached to the heart and isolate the heart.

m. Mount the heart by fixing the cannula in screw clamp.

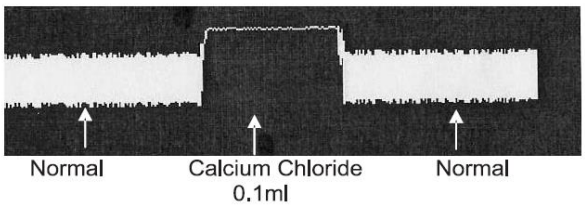
n. Attach one end of thread to the lower part of the ventricle and other end of thread to the Starling's heart liver.

o. Adjust the tip of starling heart liver on the drum fixed to Sherrington's rotating machine for recording the response.

p. Record the normal heart beat on the paper of the drum.

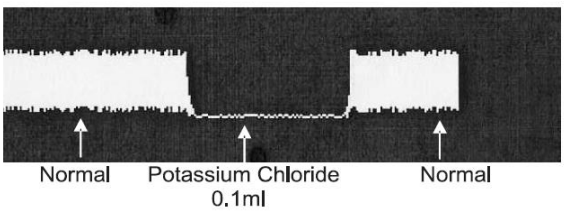
q. Inject the doses of 0.1 ml of calcium chloride solution into Syme's cannula.

r. Immediately switch on the kymograph and record the effect of ACh for 2 minutes period. After 2 minutes switch off the kymograph till the heat beat and amplitude comes to the normal.



s. Again record normal response of heart and inject 0.2 ml of calcium chloride and repeat step 18.

t. Similarly give injection of 0.1 ml and 0.2 ml of potassium chloride and repeat the steps and record the responses.



u. After completion of doses, label and fix the graph with fixing solution.

v. Observe the response of calcium and potassium ions, and record in observation table.

8. Observations:

Record the observation from the Pharmacology software in the following table. Observe and record heart rate, force of contraction and tone.

Sr. No.	Dose of Calcium chloride (ml)	Heart rate (Beats/min)	Force of contraction	Tone
1	Frog ringer	30-50	Normal	Normal
2	0.1 ml			
3	0.2 ml			

Sr. No.	Dose of Potassium chloride (ml)	Heart rate (Beats/min)	Force of contraction	Tone
1	Frog ringer	30-50	Normal	Normal
2	0.1 ml			
3	0.2 ml			

9. Result:

1. Calcium ion was found to increase / decrease heart rate.
2. Calcium ion was found to increase / decrease force of contraction.
3. Calcium ion was found to increase / decrease tone.
4. Potassium ion was found to increase / decrease heart rate.
5. Potassium ion was found to increase / decrease force of contraction.
6. Potassium ion was found to increase / decrease tone.

10. Conclusion:

From the result it can be concluded that calcium shows ……....and potassium shows …….. effect on isolated frog heart.

11. References:

a) Kulkarni SK. Practical Pharmacology and Clinical Pharmacy. 1st edition, Vallabh Prakashan, Delhi.

b) Badyal D. Practical manual of pharmacology. 1st edition, Jaypee brothers medical publishers, New Delhi.

12. Practical Related Questions (Teacher can give more questions to the student):

a) What is the effect of calcium ion on heart?

b) What is the effect of potassium ion on heart?

c) How calcium ion contracts heart?

d) Draw the graph of effect of calcium ions on isolated heart of frog.

e) Draw the graph of effect of potassium ions on isolated heart of frog.

f) What is the effect of excess calcium and potassium on heart?

(Space for answers)

13. Assessment Scheme:

Particular	Understanding the basic concept (Intellectual skill)	Performance of the experiment (Intellectual and motor skill)	Cleanliness & Handling (Affective domain)	Viva-voce /Answers Written	Total	Signature of teacher
Marks Obtained						
Max Marks	**02**	**05**	**01**	**02**	**10**	

Experiment No. 23
Study of effect of Cardiotonics on isolated frog heart

1. Aim:

To Study of effect of Cardiotonics on isolated frog heart

2. Practical Significance:

Cardiotonics are used in the treatment of heart failure as they have ability to increase the force of contraction (positive inotropy) of a hypodynamic heart. Hypodynamic heart can be experimentally modeled using an isolated frog heart by perfusing it with modified Frog Ringer Solution. This modified Ringer contains $1/4^{th}$ of the $CaCl_2$than the normal Frog Ringer solution. The normal Ringer contains 0.12 g/lit of $CaCl_2$ whereas, the modified Ringer used to induce a hypodynamic heart contains 0.03 g/lit of $CaCl_2$

3. Practical Outcomes (PrOs):

After completion of this practical, the students will be able to:

PrO	Practical Outcomes	Mapped CO	BTL
PrO1	Explain the working concept of hypodynamic heart.	CO 2,3	BTL2
PrO2	Describe the mechanism of action of cardiotonics.	CO 2,3	BTL2
PrO3	Handle the software for recording the responses.	CO 2,3	BTL3

4. Relevant Theoretical Background:

Heart failure

Heart failure is defined as a condition in which the cardiac output is decreased and the heart is unable to meet the body's oxygen and blood supply demands.

Cardiotonics:

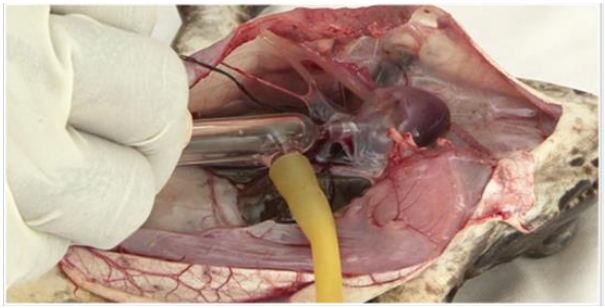
These are the drugs which increased the force of contraction of the cardiac muscles in the failing heart (during heart failure). These drugs include Na^+-K^+-ATPase inhibitors, positive inotropic agents (sympathomimetics, parasympatholytics, xanthines etc.)

Figure 23.1 Setup for the evaluation of cardiotonic activity on isolated and perfused frog heart

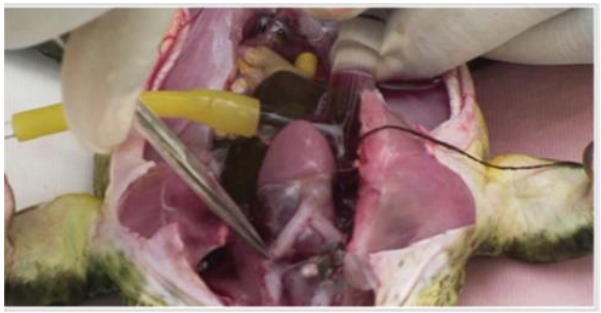
5. Precaution:

a. Prior to experimentation the frog must be pithed and the corneal and pinch reflexes must be checked

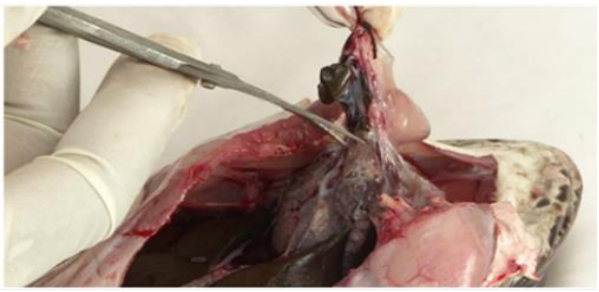
b. The sinus venosus should be traced and cannulation should be done at sinus venosus



c. After cannulation, the carotid must be dissected to allow outflow of Ringer solution

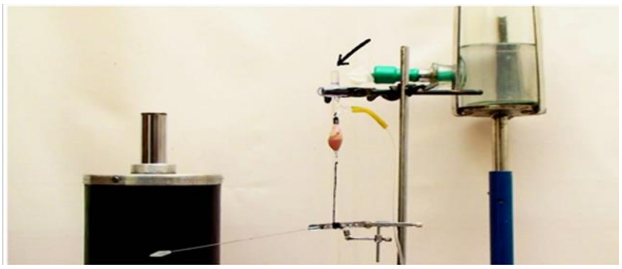


d. Carefully isolate the heart without piercing the atria



e. As frog is a cold-blooded animal, there is no need of adjusting the temperature of the Frog Ringer solution

f. Add the drug solution to cannula at the below indicated arm of cannula



6. Requirements:

Animal: Frog

Perfusate: Frog Ringer Solution, Modified frog Ringer Solution (containing $1/4^{th}$ $CaCl_2$)

Drugs: Digoxin (10 mg/ml)

Apparatus and Equipment: Symes cannula, Marriotes bottle, Startling's Heart lever sherrington's rotating drum machine

7. Procedure:

a. Isolation and mounting of frog heart

Holding the frog: Hold the frog in such a way that the thumb of left hand is pressed against its back. The right front leg of the frog is held between index finger and middle finger of the left hand while rest two fingers are on its back. Left front leg and hind legs of the frog are free.

b. Position for pithing: Pithing is done at the junction between cranium and atlas vertebra (this relates to the foramen magnum). The position of foramen magnum is decided by sliding the pithing needle along the midline on frog's head. Pithing has to be done at the point where the first slight depression is felt.

c. Pithing: Insert a sharp needle in the foramen magnum towards the brain and destroy a part of it. Then remove and reinsert the needle in opened spinal canal and destroy a part of the spinal cord by inserting the needle backwards. This may cause the frog to urinate and throw its hind legs in convulsion.

d. Checking the reflexes: To check whether the frog has been properly pithed, touch the cornea of eye with the needle and see whether corneal responses have completely subsided. Also, 'touch and pain' reflexes can be checked by superficially pricking the hind leg of the frog to see whether jerking movement occurs. A properly pithed frog shows neither corneal nor pain reflexes.

e. Lay the pithed frog on its back. With a fine scissors, take a small 'V' shaped cut in the abdominal skin at the pelvic girdle. Insert a curved scissors in this 'V' shaped cut and cut the abdominal skin up to the pectoral girdle.

f. The underlying muscular part shows rectus abdominis muscle. Take a bold cut on one side of the central vein. Through this cut, insert the blunt side of the scissors and take a cut up to pelvic girdle without injuring the visceral organs.

Cut the pelvic girdle with a bone cutter or larger scissors to expose the heart.

g. Remove the Pericardium with the help of a blunt forceps to avoid any injury to the heart. With the thumb of left hand push upwards the ventricle of heart and locate the sinus venosus.

h. Start a weak flow of P. S. S. through the cannula. Insert the cannula in the central vein of sinus venosus through the cut and tie it in position with the thread. Cut the aorta to let out the perfusate.

i. Hold the cannula between the index finger and the middle finger of left hand and slightly lift it up. Carefully cut off the tissues attaching to the heart with a scissors and isolate the perfused heart.

j. Mount the isolated heart on the stand as shown. Superficially insert the pin Attached to Starling's lever, in the wall of ventricle at its tip. Adjust lever to make it horizontal.

k. After mounting the heart, using normal Ringer solution, start the flow of the modified Ringer and check whether the force of contraction (amplitude of recording) of heart gets lowered.

l. Once, a lowered force of contraction is recorded, initiate the dosing with Digoxin as 01. ml, 0.2 ml, 0.3 ml etc. and record the heart activity. Count the heart rage and check how the force of contraction changes

8. Observations:

Record the observation from the Pharmacology software in the following table.

Sr. No.	**Treatment**	Heart rate (beats / minute)	Force of contraction (increased/ decreased)
1	Normal Ringer		
2	Modified Ringer		
3	Digoxin 0.1 ml		
4	Digoxin 0.2 ml		
5	Digoxin 0.3 ml		

9. Result:

Digoxin ……heart rate and ……..force of contraction in a dose dependent manner.

10. Conclusion:

From the above observations it is concluded that Digoxin exerts cardiotonic effect in a hypodynamic heart.

11. References:

a) Kulkarni SK. Practical Pharmacology and Clinical Pharmacy. I edition, Vallabh Prakashan, Delhi.

b) Goyal RK. Practicals in Pharmacology, B. S. Shah Prakashan.

12. Practical Related Questions (Teacher can give more questions to the student):

a) What is mechanism of action of Cardiotonics?

b) Write classification of drugs used in treatment of heart failure

c) What modifications is done in the composition of Ringer to induce a hypodynamic heart?

d) What is the effect of calcium and potassium concentration on the effects of digitalis?

e) Define and write the examples of Cardiotonics.

(Space for answers)

13. Assessment Scheme:

Particular	Understanding the basic concept (Intellectual skill)	Performance of the experiment (Intellectual and motor skill)	Cleanliness & Handling (Affective domain)	Viva-voce /Answers Written	Total	Signature of teacher
Marks Obtained						
Max Marks	**02**	**05**	**01**	**02**	**10**	

Experiment No. 24
Effect of drugs Parasympathomimetic drugs on ciliary motility on frog's oesophagus

1. Aim:
To Study of the effects of Parasympathomimetic drugs on the ciliary motility on frog oesopahgus.

2. Practical Significance:
Parasympathomimetics are the agents which exert acetylcholine-like effects. These can be directly acting agents (acetylcholine) or indirectly acting (through inhibition of the acetylcholinesterase activity).

Cilia are a hair like projections lining the cells present on the surface of the mucus membranes. The cilia continuously keep on moving (ciliary movement) to either remove the mucus or any foreign particles deposited on the mucus membranes. Such cilia are present on epithelial lining of the lumen of oesophagus and their continuous beating removes the mucus from the inner surface of the oesophagus.

Parasympathomimetic agents increase the ciliary motility by acting on the muscarinic receptors. This increased movement of cilia can be detected using poppy seeds and the rate at which these poppy seeds move on the surface of the exposed lumen of the oesophagus.

3. Practical Outcomes (PrOs):
After completion of this practical, the students will be able to:

PrO	Practical Outcomes	Mapped CO	BTL
PrO1	Explain the working concept of effects of Parasympathomimetics on ciliary motility	CO 2,3	BTL2
PrO2	Describe the mechanism of action of Parasympathomimetics	CO 2,3	BTL2
PrO4	Handle the software for recording the responses.	CO2, 3,4	BTL3

4. Relevant Theoretical Background:
Parasympathomimetics

These are the agents which exert either direct agonistic actions on the cholinergic receptors or inhibit the action of acetyl cholinesterase enzyme and thereby inhibit the degradation acetylcholine in the synaptic regions.

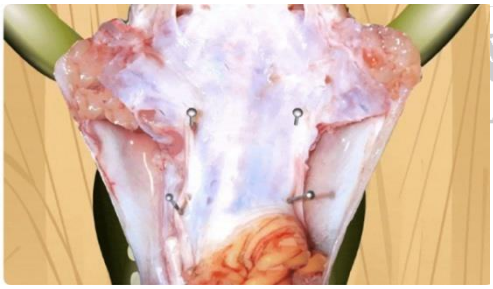


Figure24.1 Setup for the evaluation of effects of Parasympathomimetics on the ciliary motility

5. Requirements:

Animal: Frog

Perfusate: Frog Ringer Solution

Drugs: Acetylcholine (10 µg/ml)

Apparatus and Equipment: Stop watch, Poppy seeds, pipettes

6. Precaution:

a. Prior to experimentation the frog must be pithed and the corneal and pinch reflexes must be checked

b. The surface of the oesophagus should be wiped frequently with a cotton swab soaked in Ringer to remove mucus and keep the surface moistened

c. Wiping should be gentle so as not to damage the cilia.

d. The surface should not be flooded with ringer.

e. Mucus hinders the movement of the seed

f. As frog is a cold-blooded animal, there is no need of adjusting the temperature of the Frog Ringer solution

g. Take separate control readings for each drug by adding the ringer on the oesophagus

h. Use a new from each drug.

7. Procedure:

a. Pithing of frog.

Holding the frog: Hold the frog in such a way that the thumb of left hand is pressed against its back. The right front leg of the frog is held between index finger and middle finger of the left hand while rest two fingers are on its back. Left front leg and hind legs of the frog are free.

b. Position for pithing: Pithing is done at the junction between cranium and atlas vertebra (this relates to the foramen magnum). The position of foramen magnum is decided by sliding the pithing needle along the midline on frog's head. Pithing must be done at the point where the first slight depression is felt.

c. Pithing: Insert a sharp needle in the foramen magnum towards the brain and destroy a part of it. Then remove and reinsert the needle in opened spinal canal and destroy a part of the spinal cord by inserting the needle backwards. This may cause the frog to urinate and throw its hind legs in convulsion.

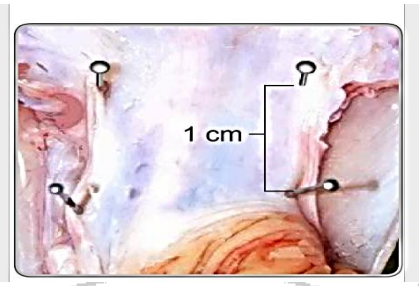
d. Checking the reflexes: To check whether the frog has been properly pithed, touch the cornea of eye with the needle and see whether corneal responses have completely subsided. Also, 'touch and pain' reflexes can be checked by superficially pricking the hind leg of the frog to see whether jerking movement occurs. A properly pithed frog shows neither corneal nor pain reflexes.

e. Lay the pithed frog on its back.

f. Remove the lower jaw of the frog using a bone cutter

g. Expose the oesophagus and take a central cut along the length of the oesophagus to expose the lumen of the oesophagus

h. Fix the opened oesophagus using pins placed exactly at 1 cm distance as shown in the figure below.



i. First add the 0.1 ml of ringer on the exposed oesophagus and place poppy seed on the oesophagus near the pin placed towards the head of the frog.

j. When the poppy moves up to the pin start the stop-watch and count the time required by the seed to reach the lower pin (i.e. time required for the seed to move a distance of 1 cm)

k. Repeat the procedure thrice for each drug solution

8. Observations:

Record the observation from the Pharmacology software in the following table.

Sr. No.	Treatment	Time (Sec)
1	Normal Ringer	
2	Normal Ringer	
3	Normal Ringer	
Average		
5	Acetylcholine	
6	Acetylcholine	
7	Acetylcholine	
Average		

9. Result:

Acetylcholine-treated oesophagus required ……seconds for the movement of the seed across 1 cm distance as compared to …….seconds required after the normal Ringer solution treatment.

10. Conclusion:

From the above observations it is concluded that Parasympathomimetics exert increase in the ciliary movement in oesophagus

11. References :

a) Goyal RK. Practicals in Pharmacology, B. S. Shah Prakashan.

12. Practical Related Questions (Teacher can give more questions to the student):

a) What is meant by cilia? What is their physiological function?

b) Write classification of Parasympathomimetics.

c) Write the action of acetylcholine.

d) Why acetylcholine is not used therapeutically?

e) Write the mechanisms of action of parasympathomimetics.

(Space for answers)

13. Assessment Scheme:

Particular	Understanding the basic concept (Intellectual skill)	Performance of the experiment (Intellectual and motor skill)	Cleanliness & Handling (Affective domain)	Viva-voce /Answers Written	Total	Signature of teacher
Marks Obtained						
Max Marks	**02**	**05**	**01**	**02**	**10**	

Experiment No. 25

Effect of drugs Atropine (Parasympatholytic drug) on the ciliary motility on frog's oesophagus

1. Aim:

To Study of the effects of Atropine on the ciliary motility on frog oesopahgus.

2. Practical Significance:

Atropine:

Cilia are a hair like projections lining the cells present on the surface of the mucus membranes. The cilia continuously keep on moving (ciliary movement) to either remove the mucus or any foreign particles deposited on the mucus membranes. Such cilia are present on epithelial lining of the lumen of oesophagus and their continuous beating removes the mucus from the inner surface of the oesophagus.

Parasympatholytic agents reduce the ciliary motility by acting on the muscarinic receptors. This reduced movement of cilia can be detected using poppy seeds and the rate at which these poppy seeds move on the surface of the exposed lumen of the oesophagus.

3. Practical Outcomes (PrOs):

After completion of this practical, the students will be able to:

PrO	Practical Outcomes	Mapped CO	BTL
PrO1	Explain the working concept of effects of Parasympatholytics on ciliary motility	CO 2,3	BTL2
PrO2	Describe the mechanism of action of Parasympatholytics	CO 2,3	BTL2
PrO4	Handle the software for recording the responses.	CO2, 3,4	BTL3

4. Relevant Theoretical Background:

Parasympatholytics

These are the agents which blocks cholinergic receptors in the effector organ supplied by cholinergic nerves.

Atropine reduces tone and motility of gastrointestinal tract. It completely abolishes excessive motility of gastrointestinal tract induced by parasympathomimetic agents. It is only partially effective in blocking the effect of vagus nerve stimulation and it dose not interferes with normal peristalsis.

Ex., Atropine, Hyoscyamine and Scopolamine.

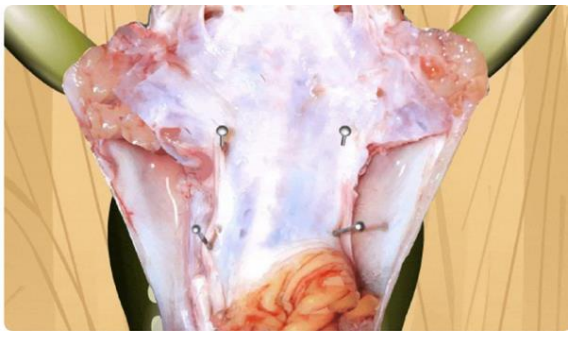


Figure 25.1 Setup for the evaluation of effects of parasympathomimetics on the ciliary motility

5. Requirements:

Animal: Frog

Perfusate: Frog Ringer Solution

Drugs: Atropine (50µg/ml)

Apparatus and Equipment: Stop watch, Poppy seeds, pipettes

6. Precaution:

a. Prior to experimentation the frog must be pithed and the corneal and pinch reflexes must be checked

b. The surface of the oesophagus should be wiped frequently with a cotton swab soaked in Ringer to remove mucus and keep the surface moistened

c. Wiping should be gentle so as not to damage the cilia.

d. The surface should not be flooded with ringer.

e. Mucus hinders the movement of the seed

f. As frog is a cold-blooded animal, there is no need of adjusting the temperature of the Frog Ringer solution

g. Take separate control readings for each drug by adding the ringer on the oesophagus

h. Use a new from each drug.

7. Procedure:

a. Pithing of frog.

Holding the frog: Hold the frog in such a way that the thumb of left hand is pressed against its back. The right front leg of the frog is held between index finger and middle finger of the left hand while rest two fingers are on its back. Left front leg and hind legs of the frog are free.

b. Position for pithing: Pithing is done at the junction between cranium and atlas vertebra (this relates to the foramen magnum). The position of foramen magnum is decided by sliding the pithing needle along the midline on frog's head. Pithing must be done at the point where the first slight depression is felt.

c. Pithing: Insert a sharp needle in the foramen magnum towards the brain and destroy a part of it. Then remove and reinsert the needle in opened spinal canal and destroy a part of the spinal cord by inserting the needle backwards. This may cause the frog to urinate and throw its hind legs in convulsion.

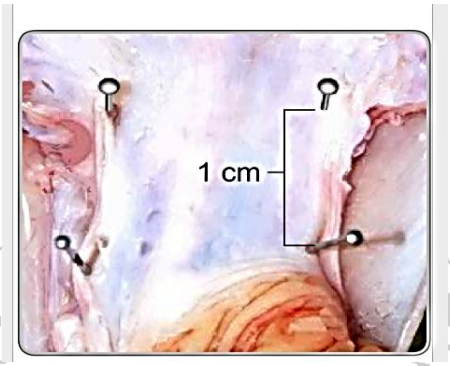
d. Checking the reflexes: To check whether the frog has been properly pithed, touch the cornea of eye with the needle and see whether corneal responses have completely subsided. Also, 'touch and pain' reflexes can be checked by superficially pricking the hind leg of the frog to see whether jerking movement occurs. A properly pithed frog shows neither corneal nor pain reflexes.

e. Lay the pithed frog on its back.

f. Remove the lower jaw of the frog using a bone cutter

g. Expose the oesophagus and take a central cut along the length of the oesophagus to expose the lumen of the oesophagus

h. Fix the opened oesophagus using pins placed exactly at 1 cm distance as shown in the figure below.



i. First add the 0.1 ml of ringer on the exposed oesophagus and place poppy seed on the oesophagus near the pin placed towards the head of the frog.

j. When the poppy moves up to the pin start the stop-watch and count the time required by the seed to reach the lower pin (i.e. time required for the seed to move a distance of 1 cm)

k. Repeat the procedure thrice for each drug solution

8. Observations:

Record the observation from the Pharmacology software in the following table.

Sr. No.	Treatment	Time (Sec)
1	Normal Ringer	
2	Normal Ringer	
3	Normal Ringer	
	Average	
5	Atropine	
6	Atropine	
7	Atropine	
	Average	

9. Result:

Atropine-treated oesophagus required ……seconds for the movement of the seed across 1 cm distance as compared to ……..seconds required after the normal Ringer solution treatment.

10. Conclusion:

From the above observations it is concluded that Parasympatholytic drug like atropine exerts inhibition of the cililary motility.

11. References :

a) Goyal RK. Practicals in Pharmacology, B. S. Shah Prakashan.

12. Practical Related Questions (Teacher can give more questions to the student):

a) What is meant by cilia? What is their physiological function?

b) Write classification of parasympatholytics

c) Give the uses of parasympatholytics.

d) Write the actions and uses of atropine.

e) Why atropine is used as Prenanesthetic medication.

(Space for answers)

13. Assessment Scheme:

Particular	Understanding the basic concept (Intellectual skill)	Performance of the experiment (Intellectual and motor skill)	Cleanliness & Handling (Affective domain)	Viva-voce / Answers Written	Total	Signature of teacher
Marks Obtained						
Max Marks	**02**	**05**	**01**	**02**	**10**	

Guidelines for conduction of Sessional Practical Examination

Subject/Abbr : Pharmacology Practical (PGP) **Max. Marks**: 80
Subject Code: 20056 **Max Time**: 3 hrs.
Year: Second year **Course code**: PH2J

Q. I. Synopsis (Give 5 questions) …… [10]

(5 questions of 2 marks each based on basic techniques (routes of administration, selection of animals, apparatus used, etc., in pharmacology practical can be asked)

Q. II. Experiments…….. [50]

a. Spotting (Give 05 spots) 10

(Completed practical can be asked)

b. Major experiment 30

Questions related to the practical (Give 15 questions)

(15 questions of 2 marks each based on practical can be asked)

c. Minor experiment 10

Questions related to practical (give 5 questions)

(5 questions of 2 marks each based on practical can be asked)

Q. III. Viva voce ……... [10]

(Viva should be conducted on practical and theory-based questions)

Q. IV. Practical Record Maintenance ……... [10]

Guidelines for conduction of Annual Practical Examination

Subject/Abbr : Pharmacology Practical (PGP) **Max. Marks**: 80
Subject Code: 20056 **Max Time**: 3 hrs.
Year: Second year **Course code**: PH2J

Q. I. Synopsis (Give 5 questions) …… [10]

(5 questions of 2 marks each based on basic techniques (routes of administration, selection of animals, apparatus used, etc., in pharmacology practical can be asked)

Q. II. Experiments…….. [60]

a. Spotting (Give 10 spots) 20

(Syme's cannula, simple canula, different types of levers, Sherrington's rotating drum, organ bath assembly, tissue holder, aeration tube, aerator, marriot's bottle, kymograph paper, hot-plate apparatus, tail-flick apparatus, EPM apparatus, wax tray, actophotometer, rota-rod apparatus, animal cage, rabbit holder, syringes, oral feeding needle, telethermometer, etc., can be asked)

b. Major experiment 30

Questions related to the practical (Give 15 questions)

(15 questions of 2 marks each based on practical on isolated tissues, pyrogen testing, evaluation of miotics, mydriatics, anesthetic, etc., can be asked)

c. Minor experiment 10

Questions related to practical (give 5 questions)

(5 questions of 2 marks each based on practical on CNS stimulants, CNS depressants, analgesics, etc., can be asked)

Q. III. Viva voce ……... [10]

(Viva should be conducted on practical and theory-based questions)

PHARMACIST'S OATH

- I swear by the code of Ethics of Pharmacy Council of India in relation to the community and shall act as an integral part of health care team.
- I shall uphold the laws and standards governing my profession.
- I shall strive to perfect and enlarge my knowledge to contribute to the advancement of pharmacy and the public health.
- I shall follow the system which I consider best for pharmaceutical care and counseling of patients.
- I shall Endeavour to discover and manufacture drugs of quality to alleviate sufferings of humanity.
- I shall hold in confidence the knowledge gained about the patients in connection with my professional practice and never divulge unless compelled to do so by the law.
- I shall associate with organizations having their objectives for betterment of the Profession of Pharmacy and make contribution to carry out the work of those organizations.
- While I continue to keep this oath unviolated, may it be granted to me to enjoy life and the practice of pharmacy respected by all, at all times!
- Should I trespass and violate this oath may the reverse be my lot!