MARRI LAXMAN REDDY
INSTITUTE OF PHARMACY

LABORATORY MANUAL

PHARMACOLOGY

II B.PHARMACY II-SEMESTER

ACADEMIC YEAR 2020-21

About MLRIP

To be an educational Institute of par excellence and produce competent pharmacy professionals to serve the community through research and the ever-increasing needs of Industry.

1. Imparting quality education and innovative research for various career opportunities.
2. Creating conducive academic environment to produce competent pharmacy professionals.
3. Indoctrination of students adorned with high human values and make them aware of their responsibility as health care professionals.

Program Educational Objectives

PEO 1: To produce graduates with sound theoretical knowledge and technical skills required for their career opportunities in various domains.

PEO 2: To incite the students towards research and to address the challenges with their innovative contributions for the benefit of the mankind.

PEO 3: To instill the essence of professionalism, ethical commitment to become a health care professional with sound integrity and adherence to the core human values in the service of the society.

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

LIST OF EXPERIMENTS FOR B. PHARMACY II/II

INTRODUCTION TO EXPERIMENTAL PHARMACOLOGY

AIM: To study general introduction of pharmacology and experimental pharmacology

DEFINITIONS:

1.Pharmacology: The word pharmacology is made of two parts, Pharmacon(drug) and logos (discourse and study), Pharmacology means study of drugs, their pharmacodynamics, pharmacokinetics and toxicities.

2.Clinicalpharmacology: The branch concerned with the scientific studies on the effects of drugs treatment in human being.

3. Pharmacokinetics: It is study of absorption, distribution, metabolism, and excretion of drugs i.e., study of what drug does to the body.

4. Pharmacodynamics: It is the study of mechanism of action and site of action of the drugs i.e., it is the study of what drug does to the body.

5. Absorption: Drug goes to the site of administration to systemic circulation or blood.

6.Distribution: Drug goes from systemic circulation to various compartments like fat, muscles, tissues, organ etc.

7. Metabolism: Conversion of drug into excretion form.

8. Elimination: Removal of drug from the body.

9. Bioavailability: Fraction of an administered dose of unchanged drug that reaches the systemic circulation.

10. Drug: It is the active ingredient which is useful for diagnosis, treatment, mitigation, and prevention of any diseases or disorder in human beings and animals.

11. **Medicine**: The substance used to deliver drug in stable and acceptable form and it consist lubricant, binder, sweetener, like other additives, constituents with active ingredient.

12. **Neuropharmacology**: Effects of medication on central and peripheral nervous system functioning.

13. **Pharmacogenetics**: Clinical testing of genetic variation that gives rise to differing response to drug.

14. **Posology**: How medicines dosed, it also depends upon various factors like age, climate, weight, sex, and so on.

15. **Pharmacovigilance**: It is defined as the science and activities relating to the detection assessment and understanding of adverse effects.

16. **Sideeffects**: A secondary but predictable effects, typically undesirable effect of a drug or medical treatment.

17. **Adverseeffects**: A secondary but unpredictable effects, typically, undesirable, effect of a drug or medical treatment.

OBJECTIVES OF EXPERIMENTAL PHARMACOLOGY

1. To screen drug substance for their biological activities.
2. To study the toxicity of drug
3. To study mechanism of action and site of action of drug

A. Preclinical experiments: which consists of animal studies for deciding the safety, efficiency.
B. Clinical experiments: These follow preclinical studies. In clinical pharmacology safety, efficiency of a drug is determined through its use in healthy human volunteers and patient populations under controlled conditions.

COMMONLY USED INSTRUMENTS USED IN EXPERIMENTAL PHARMACOLOGY

AIM: To study the commonly used instruments in experimental pharmacology

ACTOPHOTOMETER:

Actophotometer consists of photocells. These photocells are activated when the rays of lights falling on the photocells are obstructed to movement of animals crossing the path of the light beam. This cut off quantity of light beam is measured electrically. This is proportional to the movement of animals on cage. This instrument measures the active exploratory movement of animal. This equipment is used to measure the effect of drug on motor activity of the rat/mice, and useful in screening and evaluation of drug for pharmacological and toxicological experiments.

ACTOPHOTO METER

ROTARODAPPARATUS:

The Rota rod performance test is a performance test based on a rotating rod with forced motor activity being applied, usually by a rodent. The best measures parameters as riding time or endurance. Some of the functions of test include evaluating balance, grip strength and motor coordinating of the subjects, especially in testing the effect of experimental drugs or after traumatic brain injury.

In the test a rodent is placed on a horizontally oriented, rotating cylinder(rod) suspendent above a cage floor, which is low enough not to injure the animal, but high enough to induce avoidance of fall. Rodents naturally try to stay on rotating cylinder, or rotarod, and avoid falling to the ground, thelength of time that a given animal stay on the rotating rod is a

measure of their balance, coordination, and motor planning. The speed of the Rota rod is mechanically driven, and may either be held constant or accelerated.

A human analog to Rota rod test might be tread mill running. Hamster, gerbil, mouse, owners can observe the principle in action when animal climbs on outside of its wheel.

ELECTRO-CONVULSOMETER:

Seizures are produced by electrically stimulation and their phases are then antagonized by systemic administration of an anticonvulsants, Different types of epilepsy can be studied in the laboratory animals. The maximal electroshock induced convulsion drug into the laboratory animals. The MES convulsions are divided into fire phases tonic fission, tonic extensor, clonic convulsion.

EDDY'S HOTPLATE:

In the hotplate we use as the Min stimulants or source of pain. Mice are the choice of animal for this experiment hotplate is a device having boundary or outer side and a plate which get heated by a heating-coil from inside. Mice are placed one by on this apparatus which temperature is kept constant by a regulator, analgesic increases the time. Eddy and Leimbach is also known as Eddy's hot plate.Eddy's Hot plate consisting of 30x30 cms. Heating surface with perpex enclosure and solid-state temperature controller with micro-controller based digital temperature indicator controller to set surface temp.A constant temperature (550C) was maintained and the reaction of animals, such as paw licking/jumping response was taken as end point.Eddy's hot plate test is used to assess analgesic activity of drugs.

ANALGESY-METER

The force applied to the paw by the plinth increases at a constant rate, thus enabling perfect reproducible measurements to be made. The motor stops immediately the pedal is released. After each test the slide should be returned to its starting point by lifting it and pushing it to the left. The force is measured on the scale calibrated in 10-gram steps, by a pointer reverted to the slide. The scale can be multiplied by 2 or 3, by placing on the slide one or two discs provided with the standard package.To evaluate antinociceptive profile of drug or mechanical pain threshold.

ELEVATED PLUS MAZE

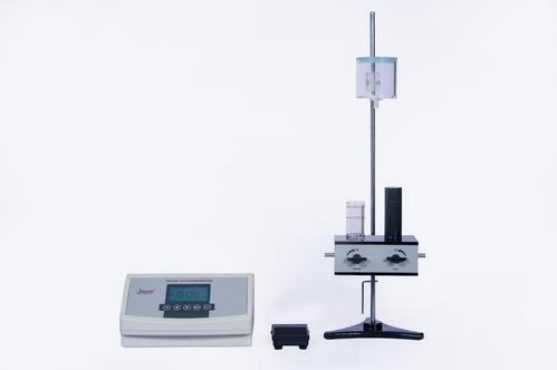
The test setting consists of a plus-shaped apparatus with two open and two enclosed arms, each with an open roof, elevated 40–70 cm from the floor. The model is based on rodents' aversion of open spaces. This aversion leads to the behavior termed thigmotaxis, which involves avoidance of open areas by confining movements to enclosed spaces or to the edges of a bounded space. In EPM this translates into a restriction of movement to the enclosed arms

Anxiety reduction in the plus-maze is indicated by an increase in the proportion of time spent in the open arms (time in open arms/total time in open or closed arms), and an increase in the proportion of entries into the open arms (entries into open arms/total entries into open or closed arms). Total number of arm entries and number of closed-arm entries are usually employed as measures of general activity.

PLETHYSMOMETER

Plethysmometer is a volume meter that has long been the standard instrument for measurement of rodent paw volume.

The paw or object to be measured is inserted into water in a clear acrylic cell, up to a planned point, such as the wrist joint. The volume of water displaced is measured by a transducer. The specially designed transducer features automatic correction for changes in conductivity of the water due to contaminants introduced by successive measurements. Thus, the measurements remain accurate and reliable even through a mass screening.



Used to screen potential anti-inflammatory or anti- edema agents, or to determine if a drug causes inflammation as a side effect.

SHERRINGTON ROTATING DRUM

Student Kymograph consists of special heavy-duty motor, oil lapped gear systems, speeds from 0.12mm/sec to 640 mm/sec, in 8 steps, jerk free running, instantaneous start & stop clutch. Stainless steel spindle with screw lift 15 x 15cm cylinder with leveling screws, pilot lamp, plug & cord.Used to record the response during bioassay of drugs.

STUDENTS ORGAN BATH

A Perspex organ bath consists of fine thermostatic temperature control. Complete with accessories, bath size 8" x 4" x 5" with leak proof dovetailed joints, fitted on sturdy metal frame.Used to study bioassay of drugs

POLE CLIMBING APPARATUS

Pole climbing apparatus is the new improved model built-in solid-state buzzer and stimulator to provide electrical shocks of 16 - 200 V DC in pulsating rates 0.1 mA at a frequency of 5 Hz for duration controlleddigitally or by built in digital selectable second timer. Output available for recording on Kymograph or polygraph complete to work on 230 V AC.

Features are Digital Voltmeter: 16 - 200 V DC. Digital Timer: 0.1 - 999 sec. Digital Delay Timer: 0.1 - 999 sec (cyclic). Complete Chamber and Tray made of thick imported Acrylic Sheets. Climbing Pole of Bakelite. Switch for selecting light or sound mode.

Learning was assessed by measuring the number of times the rat climbed the pole after the conditioned stimulus to avoid the foot-shock unconditioned stimulus.

STUDY OF COMMON LABORATORY ANIMALS

MAINTENANCE OF LABORATORY ANIMALS AS PER CPCSEA GUIDELINES

INTRODUCTION: Rats and mice are the most commonly used animals in experimental pharmacology they are usually submissive in nature. The animals should be handled carefully and restrained in correct way to avoid an injury. Good and careful handling of animals also reduces stress caused by manipulation. This helps to reduce aggressive behavior of the animals. Regular handling of the animals increases its similarity with the experiment. Careless handling and poor conditions increase the zoonotic disease i.e., the diseases transmitted from animals to human beings, there is a risk of developing allergy, conjunctivitis and skin rashes of different types. Rare cases of anaphylactic shock due to rat bite have been reported.

The source of allergens is usually urine and therefore urine-soaked bedding should be removed immediately to avoid the allergens. It is therefore necessary to protect nostrils adequately. Hands must be washed with disinfectant before leaving the animal house.

In certain circumstances as usual way because more difficult to handle and this can increase the risk of injury, to either as usual or to the experiments. Animals precured from another source should be handled carefully. The rat or mice kept in isolating are usually very aggressive. Animals should be handled frequently falling which they become difficult to handle.

The animals should be captured to its removal the cage. It is convenient to remove animals by holding the base of the tail. The animals can be handled by their tail only for removing from cage and shifting animals from one place to another within short time. The animals should not be suspended in the air for more than 2-3 sec or else become aggressive. Its body must be supported while moving from one place to another.

Rats and mice have loose skin on their bodies. The rat can be captured by gripping the base of tail and by placing the other hand on back, with the

help of thumb and forefingers, hold over the neck. Before injecting the needle or inserting the gauge cannula for oral administration, it is necessary to see that arrival is comfortably restrained and does not make attempts to escape. This avoids injury to animals.

AIM: Introduction for animals used for experimental pharmacology.

INTRODUCTION: Since long animals' experiments have been a mild stone in advanced medical research or every animal is suitable for experimental pharmacological experiments. Their section is based on the following criteria.

1. **Size**: smaller animals are preferred because they are easy to handle and less quantity of the drug is required
2. **Availability**: animals which are commonly available should be selected e.g., frogs, rats, rabbits and mice
3. **Sensitivity**: animals which are sensitive to drugs under trails, e.g., guinea pig is sensitive to effect of histamine
4. **Species**: In rabbits, intracerebral ventricular injection of 5-HT induces a lowering of temperature

-Guinea pigs and humans are 500 times more sensitive to histamine than our rat and mice

-The rat heart is known to be very resistant to cardiac glycosides

COMMONLY USED EXPERIMENTAL ANIMALS:

1. **MOUSE: (Mus musculus)**
   Suriss albino mice are commonly used. They are similar, smaller, cheap and easy to handle
   Common behavior:
   - Timid
   - Social
   - Territorial
   - Nocturnal
   - Rarely Aggressive when handled properly

Experimental use:Adult weight- 20 to 25 gm; age suitable for experiments is 2 months)

- Toxicology studies especially acute and sub acute toxicities
- Bioassay of insulin
- Screening of analgesic
- Study related to genetic and cancer research

2. **RAT: (Rattus norvegious)**

Albino rats of Wister strain are commonly used. Other strains used are Sprague dawely are commonly used. Advantageous and characteristics of rats.

1. It is small in size compared to other animals so drugs are required in small quantity
2. Vomiting center is absent so drug can be administered orally
3. Gall bladder and tonsils are absent
4. Pancreas is diffused, therefore difficult to produce pancreatomy
5. In stomach fundus and pylorus parts have clear lining between them

EXPERIMENTALUSEOFRATS: (Adult weight – 200 to 250 mg and the age suitable for the experiments is 1.5 months)

- Psychopharmacology studies
- Study of analgesic and anticonvulsant
- Study of oestrus cycle
- Gastric acid secretion and study
- Chronic study on blood pressure

3.GUINEAPIG: (**purcellus**)

It is a docile animal, highly susceptible to TB and anaphylaxis, it is highly sensitive to histamine, penicillin.

EXPERIMENTAL USE: (Adult weight is 400 to 600 gm; age suitable for experiment is 2 months)

- Evaluation of bronchodilators

- Immunological studies
- Study of ostreus cycle
- Study on mast cells
- Chronic study on mast cells

4 **RABBIT**: (Adult weight is 1.3 to 2 kgs)

- Pyrogenic testing
- Bioassay of antidiabetic
- Irritancy test
- Pharmacokinetics study
- Study of local anesthetics
- Screening of embryo genomic

5. HAMSTER:(Criticulus griscous)

They have short body with the legs and tails. The skin is loos and covered with dense, short, soft, fur. The cheek pouches are prominent and extended up to shoulder region.

EXPERIMENTAL USE: (Adult weight- 80 to 9o gm age suitable for experiment is 1 month)

- Chinese hamster has 1000 chromosomal number, making is useful for cytological investigation
- Bioassay of prostaglandins
- Research on diabetes mellitus

6. FROG (Rana tigrina)

This is one of the common animals used physiology, toxicology and pharmacology. It has been used in experiments for more than 200years. It is an amphibian animal safe to handle, it cannot be breed in lab, adrenalin in neurotransmitter in sympathetic system.

EXPERIMENTAL USE:

- Study of isolated tissue such as rectus abdominous muscle, heart tissue
- Study of drugs acting on CNS

- Study of drugs on neuromuscular junctions

Table 1. Some physiological data of laboratory animals

PARAMETER	MOUSE	RAT	GUINEA PIG	RABBIT
Biological Source	*Mus Musculus*	*Rattus Norvegicus*	*CaniaPorcellus*	*Oryctolagus cuniculus*
Order	*Rodentia*	*Rodentia*	*Rodentia*	*Laggomorpha*
Family	*Muridae*	-	-	*Leproridae*
Life span	*1-3 yrs*	*2-3yrs*	*2-6yrs*	*3-7yrs*
Adult weight (Male)	*20-40 gm*	*350-400gm*	*1000-1200gm*	*4-5 kg*
Adult weight (Female)	*18-40 gm*	*180-200gm*	*850-900gm*	*4-6 kg*
Surface area	*0.03-0.06 cm^2*	*0.03 to 0.06 cm^2*	*0.03 to 0.06 cm^2*	*1.270-3.040 cm^2*
Chromosome no (diploid)	*40*	*42*	*64*	*44*
Food consumption	*4-5 g/day*	*10-20g/day*	*20-30g/day*	*50g/day*
Water consumption	*5-8ml/day adlibitum*	*20-30ml/day ad libitum*	*12-15ml/day ad libitum*	*50-100sml/day ad libitum*
Body temperature	*36.5°c*	*37.5°c*	-	-
Oxygen consumption	*1.69 ml/g/hr*	-	-	-
Breeding Season	*continuous, cyclic*	*continuous, cyclic*	*continuous, cyclic*	*all year*
Estrus cycle	*4-5 days*	*4-5days*	*16-18 days*	
Gestation Period	*17-21 days*	*20-22 days*	*59-70 days*	*29-35 days*
Litter size	*12 pups*	*10-12pups*	*1-8 pups*	*4-10 pups*
Birth weight	*1.5gm*	*5-6gm*	*90-120 gm*	*30-100 gm*
Age at Weaning	*16-21 days*	*21days*	*21-28 days*	*4-6 weeks*
Heart rate	*320-800 beats/min*	*250-500 beats/min*	*240-277 beats/min*	*250-300 beats/min*
Blood pressure	*133 to 160mm Hg(systolic) 102 – 110mm Hg(diastolic)*	*116 to 145mm Hg(systolic) 76 – 97mm Hg(diastolic)*	*70 to 82mm Hg(systolic) 42 to 54mm Hg(diastolic)*	*70 to 82mm Hg(systolic) 42 to 54mm Hg(diastolic)*
RBC lifespan	*20 to 30 days*	-	-	-
RBC diameter	*6.6 microns*	-	-	-
Plasma PH	*7.2 to 7.4*	-	*7.35*	*7.35*
Respiration Rate	*163/min (84-230/min)*	*80-150/min*	*69-160/min*	*35-55/min*
Tidal volume	*0.18ml (0.09-0.38ml)*		*1.8 ml*	*1.8 ml*

Minute Volume	*24ml/min (11-36ml/min)*	*-*	*0.16 ml/min*	*0.16 ml/min*
Mating systems	*1:1 or 1 male to multiple females*	*1:1 or 1male to multiple females*	*1:1 or 1male to multiple females*	*1.1 or artificial*
Blood volume	*6 -7% of body weight (plasma45ml/kg , whole-78ml/kg)*	*6 - 7% of body weight*	*6 - 7% of body weight*	*6% of body weight*
RBC count	*7 to 12 × 106/mm3*	*6-10X10^6/mm^3*	*4.5-7X10^6/mm^3*	*4.5 - 7X10^6/mm^3*
WBC count	*3 to 12 × 103/mm3*	*7-14X10^3/mm^3*	*5-15X10^3/mm^3*	*5-12X10^3/mm^3*
Platelet count	*1000 to 1600 × 103/mm3*	*800-1500X10^3/mm^3*	*250-50X10^3/mm^3*	*250-750X10^3/mm^3*
Urine PH	*6.0-7.5*	*6.0-7.5*	*6.0-7.5*	*8.2*
Urine volume	*1-3 ml/day*	*10-15ml/day*	*10-15ml/day*	*50-130ml/day*
Hemoglobin	*13-17gm/dl*	*11-18g/dl*	*11-17g/dl*	*11-14g/dl*
Mean corpuscular volume	*43 to 54*	*50-65*	*50-65*	*58-72*
Mean corpuscular Hb	*13-18*	*-*	*-*	*18-24*
Mean corpuscular Hb concentration	*31-34*	*32-38*	*32-38*	*30-35*

7. CAT:(Mongreal)

Bagel dogs are used. It is easily available in large size animals. Dogs can be easily trained and tamed. It has small stomach and short intestinal and resembling those together inseparable in some trunk.

EXPERIMENTAL USE:

- Gastric acid secretion
- Acute experiment for drugs effecting blood pressure intestinal movements
- Study of antidiabetic agents
- Pharmacokinetic studies

STRAIN	DESCRIPTION	STRAIN	DESCRIPTION

CD-1 mice	*Outbred albino strain descended from swiss mice.*	***Sprague Dawley (Sd) Rats***	*Outbred albino strain originated by R. W. Dawley team hybrid hooded male and female wistar rat.*
CF-1 mice	*Outbred albino strain not descended from swiss mice.*	***Wistar Rats***	*Outbred albino strain originated at the wistar institute.*
Swiss-Webster mice	*Outbred albino strain from selective in breeding of swiss mice by Dr. Leslie Webster.*	***Long-Evans Rats***	*Outbred white with black or occasional brownhood, originated by Drs Long and Evans by cross of white wistar female with wild gray male.*
SKH1 (hairless) mice	*Outbred strain that originated from an uncharacterized strain.*	***Zucker Rats***	*Outbred obese strain with four principal coat colors (predominantly brown, brown white, predominatly black or black+white.*
BALB1C mice	*Inbred albino strain developed originally from H.J Bagg (Bagg albino).*	***Fischer 344 (F-344 Rats)***	*Inbred albino strain originated from mating 344 of rats obrained from local breeds (Fischer).*
C3H mice	*Inbred agouti strain developed originally from 'Bagg albino' female & DBA male.*	***Lewis Rats***	*Inbred albino strain originally developed by Dr Lewis from wistar stock.*
FVB mice	*Inbred albino strain derived originally from outbred swiss colony.*	***Wistar Kyoto Rats***	*Inbred albino strain originated from a brown mutation in a stock of rats trapped from Kyoto school of medicine*
AKR mice	*Inbred albino srain originally developed by Furth as a high leukemia strain.*	***Brown Norway Rats***	*Inbred non-angouti brown strain originated from a brown mutation in a stock of rats trapped from the wistar institute in 1930.*
B6C3F1 mice	*Hybrid agouti strain from female C57BL16W×male C3H/He*	***Spontaneous Hypertensive (Shr) Rats***	*Inbred albino strain developed from wistar Kyoto rats with spontaneous hypertension*
NudeCD-1 mice	*Outbred hairless albino strain that is athymic & thus immunodeficient (unable to produce T-cells)*		

8. MONKEY:

Monkey, Apes are the primates belonging to highest order of mammals, the anatomy and physiology of the monkeys and apes closely resemble to that of men. The studies done on monkey may directly translated to humans considering human expecting human tests in primates should be done only in last stage on evaluation of drug.

COMMON LABORATORY TECHNIQUES

a. ANESTHESIA IN EXPERIMENTAL ANIMALS

AIM: To study the anesthesia of experimental animals.

DESCRIPTION: Anesthesia: Loss of sensation, usually by damage to a nerve or receptor, loss of the ability to feel pain, caused by administration of a drug or other medical intervention.

Analgesia: Relief from pain

Tranquilization: A state of behavioral change in which the animal is relaxed, unconcerned by its surroundings and often indifferent from pain.

Sedation: Mild state of CNS depression in the animal is awake and calm Local Anesthesia: Loss of sensation in limited area. Regional Anesthesia: Insensibility in a larger but still limited area.

Basal Anesthesia: A light level for general anesthesia produced by a pre-anesthetic agent which prepares the animal for deeper anesthesia/ administration of other agents.

General Anesthesia: Complete loss of consciousness. Surface anesthesia: A level of consciousness accompanied by muscular relaxation to a degree that allows painless surgery. E.g. Tetracaine and propiocaine

Injectable local anesthetics: Procaine, lidocaine, mepivacaine and etiodocaine.

♣ Only recommended for gentle and calm animals (cattle, sheep)

♣ Most laboratory animals, general anesthesia is the method of choice.

In biomedical research, experiments should only be done with a conscious animal, if not possible to do the study in a anesthetized one. Anesthetic conditions should always be chosen to exclude stress, discomfort and pain which could have negative influences on the pharmacological results and reproducibility of the data.

GENERAL ANESTHESIA

Preparation: Animal should be fasted for 12 hrs Water- fasting-ad libitum

Premedication: For easier administration of the anesthetic for elimination of side effects of the anesthetic used, E.g. Atropine - Administered i.m prior to general anesthesia to avoid cardiopulmonary problems and to decrease saliva production Recommended dose: 0.05to 0.1mg/kg body wt.

Species	Premedication	Sedation	Short anaesthesia	Medium anaesthesia	Long anaesthesia
Rat	Atropine (0.2 s.c.)	Diazepam (2.5 i.m.)	Alfentanyle + Etomidate (0.03 + 2 i.m.) or Inhalation (Isoflurane)	Xylazine + Ketamine (5 + 100 i.m.) or Pentobarbitone (50 i.p.)	Xylazine + Ketamine (16 + 100 i.m.) or Urethane (1 500 i.m.)
Mouse	Atropine (0.1–0.25 s.c.)	Diazepam (5 i.p.)	Alfentanyle + Etomidate (0.03 + 2 i.m.) or Inhalation (Isoflurane)	Xylazine + Ketamine (5 + 100 i.m.) or Pentobarbitone (50 i.p.)	Xylazine + Ketamine (16 + 100 i.m.)
Hamster	Atropine (0.1–0.2 s.c.)	Diazepam (5 i.p.)	Inhalation (Isoflurane or Ether)	Xylazine + Ketamine (5 + 50 i.m.) or Pentobarbitone (35 i.p.)	Xylazine + Ketamine (10 + 200 i.m.)
Guinea pig	Atropine (0.1–0.2 s.c.)	Diazepam (2.5–5 i.m.)	Inhalation (Isoflurane)	Xylazine + Ketamine (2 + 80 i.m.)	Xylazine + Ketamine (4 + 100 i.m.) or Pentobarbitone + Chloralhydrate (30 i.p. + 300 i.v.)
Rabbit	Atropine (0.1–0.2 s.c.)	Diazepam (1–5 i.m.)	Inhalation (Isoflurane)	Xylazine + Ketamine (5 + 25–80 i.m.)	Xylazine + Ketamine (5 + 100 i.m.) or Pentobarbitone + Chloralhydrate (30 i.p. + 300 i.v.)

Xylazine – administered i.m to make the animal calm, dilates the blood vessels

COURSE OF ANESTHESIA

Four stages of anesthesia:

I. Stage of analgesia (from the first effect to unconsciousness): Heart and respiratory rate increase, normal dilation of pupils.

II. Stage of excitation: (from the beginning of unconsciousness to the start of regular respiration): Respiration irregular dilated pupils, increased motor, reflexes, nystagmus, and opisthotonus.

III. Stage of tolerance: (from the beginning of regular respiration to the termination of spontaneous respiration): narrow pupils, skeletal muscles relaxed, no eyelid reflex, corneal reflex present, flat respiration, good analgesia.

IV. Stage of asphyxia: (after termination of the spontaneous diaphragmatic respiration): No reflexes, no respiration: danger of death, immediate use of antidotes is necessary to prevent death.

Routes of general anesthesia: In general, there are two different routes to induce general anesthesia.

Injection and Inhalation anesthesia

1) **Injection**: By using this route, the narcotic compound is dissolved in a liquid. The route of administration may be i.v, i.m, s.c or i.p. The most frequently used compounds are mentioned below:

   a. **Barbiturates**- Very short acting barbiturates are used predominantly. E.g. sodium pentobarbitone, thiopental-sodium. Barbiturates are not analgesic and should not be given without opioids.

   b. **Chloral hydrate**- CV side effects are seen with this anesthetic therefore its use for laboratory animals is limited.

   c. **Ketamine**- A neuroleptic compound with a very fast onset of action following intramuscular administration. Side effect is an increased tonus of skeletal muscle but can be prevented by the simultaneous administration of xylazine.

   d. **Hypotonic agents**- These are compounds which produce a very deep sleep without analgesia. E.g. metomidate. Combination with neuroleptic agent is recommended.

   e. **Xylazine**- It is frequently used in combination with other substances.

   f.**Urethane**- Formerly used as a hypnotic agent, at appropriate dose, produces a long acting (about 10hr) anesthesia in rats. It is liver toxic, therefore limited use.

Inhalation: This kind of aesthesia plays only a minor role for small laboratory animals like rodents. It is more common for the bigger laboratory animals such as dogs, cats, monkeys, sheep and goats. The advantages of this form of anesthesia are the possibilities of controlling exactly the depth of anesthesia and of fast management of complications.

PROCEDURE: Rat anesthesia Xylazine–Ketamine

- In a sterile 10 mL bottle with a rubber stopper, mix 8.75 mL of Ketamine (100 mg/mL), and 1.25 mL of Xylazine (100 mg/mL). SHAKE WELL BEFORE USE.
- Expires in 3 months
- Keep away from light, in a cool place.
- Administer 0.05–0.10 mL/100 g IP.
- Repeat as required with 1/3 to 1/2 doses at a time (approximately every 30 minutes).
- Prevent heat loss until the animal recovers.

REPORT:

b. LABORATORY ANIMAL EUTHANASIA

AIM: To study about the laboratory animal euthanasia

DESCRIPTION:

The killing of animals used for scientific purposes is a very sensitive issue and requires special consideration to ensure that animal anxiety and fear is reduced to a minimum.

Euthanasia is defined as killing painlessly. Animals were euthanized in laboratories for the following reasons:

- At the termination of studies, to provide tissues for scientific purposes.
- When pain, distress or suffering are likely to exceed designated levels.
- Where the health or welfare of animals are grounds for concern.
- When animals are no longer used for breeding.
- When stock is not required for certain reasons, such as sex preference utilization.

OBJECTIVES OF EUTHANASIA:

To meet the objectives of euthanasia procedures must:

- Avoid distress and produce rapid loss of consciousness until death occurs.
- Be reliable, reproducible and irreversible.
- Be appropriate for age, species, and health of the animal.
- Require minimum restraint.
- Be compatible with the objectives of the study.
- Be simple to administer.
- Be safe for the operator.
- Be aesthetically acceptable to the operator, where at all possible.

TECHNIQUES OF EUTHANASIA

The techniques listed here fall within two general categories:

- Those methods that are recommended.
- Those methods that is acceptable with reservations. The reservations may be on aesthetic

grounds, the need for special equipment, or pose some possible human safety hazard. These might be used where, for example, the recommended method may impact negatively on the science, or as a second method to ensure that death has occurred.

Techniques can be further divided into:

- Chemical, which is further subdivided into: Inhalant, and injectable
- Physical

LOCATION OF EUTHANASIA AND DISPOSAL OF CARCASSES

Animals should be killed in a clean quiet environment, away from other animals where possible. Death must be established before disposal of the carcass. Records should be kept. Where practicable, tissue from animals being killed should be shared among investigators and teachers. Dependent neonates of animals being killed must also be killed or provision made for their care. If immediate disposal is not available carcasses should be frozen.

ANIMALS

Rats and Mice, Rabbits and Guinea Pigs

Rats and Mice

Recommended	Acceptable with Reservations
CHEMICALS	
• **Inhalant:** Carbon dioxide preceded by Isoflurane • **Injectable:** Pentobarbitone sodium i/p	
PHYSICAL	
Please note there are no recommended physical procedures	• Cervical dislocation (Rats heavier than 150g to be anaesthetised first) • Decapitation (may be required under special experimental design - Aesthetically unpleasant) • Stunning and exsanguination (Aesthetically unpleasant)

RECOMMENDED TECHNIQUES CARBON DIOXIDE:

Carbon dioxide, passed through a reduction valve, can be piped into plastic bags or deep containers at an optimal flow rate that displaces 20% of the chamber volume per minute. Considerable debate has occurred relative to three methods of administration of carbon dioxide for euthanasia of rats and mice:

Placing them into a container pre-filled with carbon dioxide

Placing them in air and then rapidly filling the container with carbon dioxide

Using a carbon dioxide/oxygen mixture e.g. 70%CO2/30%O2

PENTOBARBITONE SODIUM INJECTION:

Pentobarbitone sodium at a concentration of 60mg/ml given i/p at a dose rate of (200mg/Kg) produces quiet induction and death. Animals euthanized by this method should not be fed to animals or birds because of the likelihood of residues of the barbiturate being present.

It should be noted that the high concentration veterinary euthanasia pentobarbitone solutions (Greater than 300mgs/ml) may produce peritoneal irritation with pain and therefore a fast-acting local anesthetic solution should be added to the solution.

Techniques that are Acceptable with Reservations

CERVICAL DISLOCATION:

This involves holding the animal prostrate with the operators thumb and fore-finger firmly squeezing the neck, with the free hand pulling the quarters caudally. This method has traditionally been used in rodents but may be aesthetically unpleasant for the operator and if this is the case animals should be anaesthetized beforehand.

DECAPITATION:

May be considered an acceptable method of choice for some neuroscience studies where generalanesthesia may interfere with study parameters although it is generally regarded as not being aesthetic and, on those grounds, and where the science allows it cervical dislocation is generally more aesthetically acceptable.

Rabbits

Recommended	Acceptable with Reservations
CHEMICALS	
• **Inhalant:** None recommended • **Injectable:** Pentobarbitone sodium *i/v* or *i/p*	• **Inhalant:** Halothane
PHYSICAL	
Please note there are no recommended physical procedures	• Captive bolt • Neck dislocation or decapitation following anaesthesia

RECOMMENDED TECHNIQUES PENTOBARBITONE SODIUM

Pentobarbitone sodium given at a concentration of 60 mgs/ml at a dose rate of 60mgs/kg produces quiet induction and death even when given by the i/p route

although the i/v route is preferred. Again, the high concentration sodium pentobarbitone "veterinary euthanasia solutions" may be associated with irritation of the peritoneum and therefore if these are used, they should be used with fast acting local anesthetic added.

Technique Acceptable with reservations Halothane

Again, human safety concerns dictate that this must only be used in a fume cupboard.

Captive bolt

Use of a captive bolt may be an option when the use of an anesthetic is not acceptable within the scientific aims of the study. The captive bolt is applied to the head between the ears and aimed downwards so as to parallel the vertical plane of thehead. Performed correctly it results in immediate unconsciousness and immediate loss of reflexes and respiration.

Guinea Pigs

Recommended	Acceptable with Reservations
CHEMICALS	
• Inhalant: Carbon dioxide • Injectable: Pentobarbitone sodium i/p	• Inhalant: Halothane
PHYSICAL	
Please note there are no recommended physical procedures	• Cervical dislocation (Rats heavier than 150g to be anaesthetised first)

c. LABORATORY ANIMAL BLEEDING TECHNIQUES

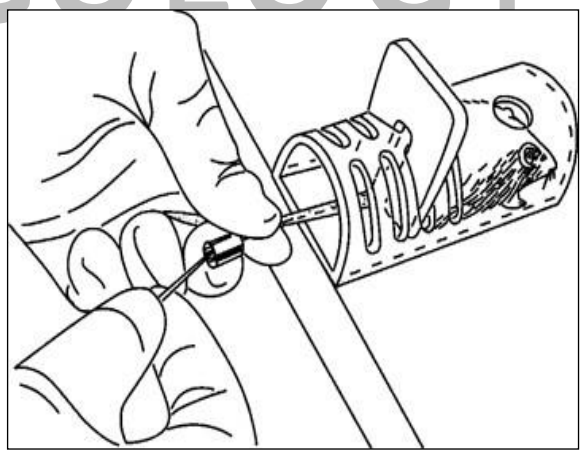
AIM: To study the bleeding techniques of laboratory animals

PROCEDURE:

Rat and mouse

Tail Vein puncture

Animal was restrained.
Lateral or dorsal veins were dilated by dipping the tail into water at 40 to 50 □C or by rubbing with xylol and then cleaned the part with some disinfectant.
The tail was grasped between the thumb and index finger.
The needle (25-27 G fitted with 1 ml syringe) was introduce near the distal portion of tail.
It was aspirated for confirmation and to avoid collapse of wall of the vein obliterating the needle opening.
Another way of collecting blood repeatedly is by cutting the tip of the tail with some sharp instrument

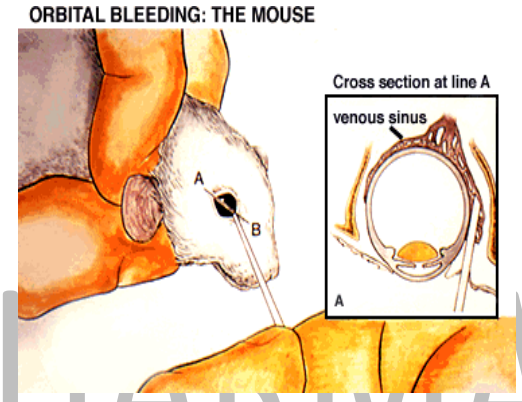


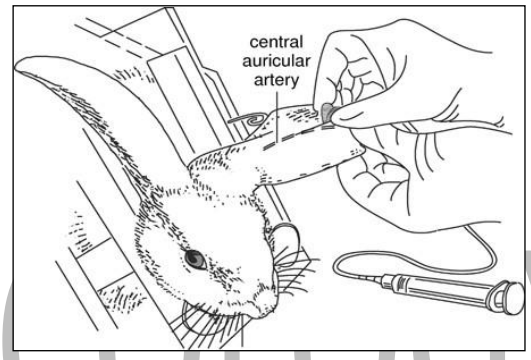
Orbital sinus venipuncture

- Animal was anaesthetized with ether.
- The capillary tube was inserted in the medial canthus with gentle rotation while directing the tube caudally and towards the midline.
- Pressure was applied after blood collection to prevent hematomas.
- 0.5 ml of blood can be obtained weekly using this method.

Rabbit

- Rabbit was restrained by using rabbit holder.
- The lateral margin of the ear was shaved and swabbed with a disinfectant.
- The ear was grasped between the thumb and the index finger.
- Lateral margin vein was punctured at a site immediately proximal to the thumb by using a 20-G needle.
- Very gentle aspiration was applied in order to avoid collapse of the vein.
- 0.5-1 ml of blood sample was collected.





HANDLING OF LABORATORY ANIMALS

AIM: To handle and restrain laboratory animals through proper technique.

PROCEDURE:

Handling of rat:

- The rat was taken out of cage gently by holding the base of the tail and placed on cage lid.
- Base of the tail was caught with right hand and slowly, the thumb and forefinger of the left hand were gradually moved up from shoulder area towards the neck muscle of rat.
- With this grip, the animal was lifted up.
- Its tail was then turned and coiled in between index and ring finger of same (left) hand.
- Finally, animal was replaced back into cage very gently.
- In-between animal was also petted by placing it on the palm.
- The gender of animal was also identified.
    a) Male: The space between anus and genital orifice was more.
    b) Female: The space between anus and genital orifice was less.

Handling of mouse:

- The mouse was taken out of the cage very gently by holding the base of the tail.
- It was then placed on the cage lid.
- Base of the tail was caught with right hand and very slowly but firmly thumb and forefinger of left hand weregradually moved up from shoulder area towards the neck muscle of mouse.
- The shoulder was pressed gently to get a better grip.
- The animal was lifted up and its tail was then

turned and coiled into left index finger.

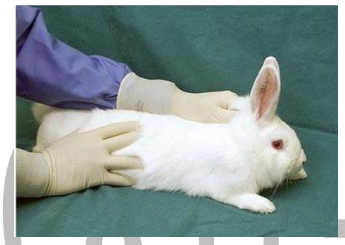
- The gender of animal was identified.
  a. **Male:** The space between anus and genital orifice was more.
  b. **Female:** The space between anus and genital orifice wasless.

Handling of rabbit:

Technique 1:

- Skin of rabbit was grasped over shoulder with one hand.
- It was gently lifted with the other arm cradling the body.Head was nested in root of arm.

Technique 2:



- The animal was held upside by the scruff off the neck with one hand.
- The animal was supported by its hind quarters with another hand.
- A towel was wrapped around the body to restrain the animal.
- Third quarters of animal were supported all the time.

Handling of guinea pig:

- The animal was restrained by grasping the whole body with thumb andforefinger around the neck.
- Other hand was placed under the hind quarters to support the whole body.
- Guinea pig was also restrained by holding against the body.

STUDY OF DIFFERENT ROUTES OF DRUG ADMINISTRATION ON MICE/RATS

AIM: To study the different routes of drug administration on mice/rats

PROCEDURE:

Intraperitoneal Injection:

- First the point of entry of needle was located. An imaginary line was drawn across the abdomen first above the knees.
- The needle was inserted along this line on the animal's right side and close to the midline.
- In case of female, the point of entry is cranial to and slightly medial to the last nipple.
- Insertion of needle on the mouse right side avoids the cecum, which is a larger fluid filled organ on the left side of the abdomen.
- Inserting the needle too for candally or laterally from the insertion point was avoided as the injection would reach the rear leg would injure the muscle must be well restrained so that it cannot move during the procedure.
- To perform an I.P. injection, the mouse must be well restrained so that it cannot move during the procedure.
- The mouse was restrained and titled so that the head is facing downward and abdomen is exposed. The needle was inserted into the abdomen at about 30 degree after disinfecting the injection site.
- The shaft of the needle should enter to a depth of about half of a centimeter. The needle was aspirated to be sure that needlehas not penetrated a blood vessel, the intestines or the urinary bladder.
- Greenish brown aspirate indicates penetration of needle into intestines.
- If any fluid was aspirated the solution is contaminated and must be discarded and the procedure was repeated with new syringe and needle
- If no fluid was aspirated the injection was given. The needle was withdrawn and the mouse was returned into the cage
- Recommended needle size I.P. is 25-27 G.

Subcutaneous Injection:

- The mouse was restrained in normal manner and skin was lifted to make a tent.The injection site was disinfected and needle was introduced into the subcutaneous tissue.
- The needle was aspirated prior to injection and when no aspirate comes indicating proper position the injection was given.
- The loose skin around the neck and shoulder area was selected for the injection site
- Subcutaneous injections were mostly used to administer fluids for hydration and to inject anesthetics.
- Typical volumes injected area of range 1ml or less
- Skin was folded over the back to form a tent.
- While injecting an awoke mouse, the mouse was place on the wire lid, so that it could hang with its front paws during theinjection.The skin over the back was scuffed and a tent was made. Hand was used both for scuffed and a tent was made both for restraining and presenting the area to be injected.
- The needle was inserted at the tents base, the needle was held parallel to the animal's body to avoid puncturing underlying structures.
- The needle was aspirated to ensure that needle has not entered a blood vessel.
- The full volume was injected at a moderate rate.
- The needle was withdrawn, skin was pressed to seal the needles exit hole and to prevent leakage of drug.
- Animal was checked for any bleeding
- Fluid was deposited in subcutaneous space and the bubble of fluid or BELB was seen and felt.
- Recommended needle size S.C. is 23-25 G.

Intramuscular injection:

- Intramuscular infections are not recommended due to lack of muscle mass in mouse.
- Injection may cause discomfort and local tissue irritation.

Intravenous injection:

- The tail vein of the mouse was used.
- The mouse was restrained with physical or chemical restraint. The tail was rotated slightly to visualize vein.
- The injection site was disinfected and needle of 27-30 G was inserted into the vein at a slight aspiration was not possible inserted injection was given slowly and cleaning of lumen was watched.
- Incorrect positioning resulted in a slight bulge in the tail, the needle was removed and process was repeated proximal to previous site, After completion the needle was removed and pressure was applied at injection site.
- Tail vein injection further details:

1. Before making an injection in a tail vein, peripheral vasodilation was induced and vein was made more prominent for cannulation
2. The body temperature of mouse was raised using an incandescent lamp, the tail was soaked in warm water or vasodilating agents such as xylazine or acepromazine was administered A safe water temperature of 110º F or 43º C was used.
3. When the needle was entered the vein, there will be less resistance to the advance of the needle inside the veins lumen than through the subcutaneous tissues.
4. Recommended needle size I.V. is 27-30 G.

Intradermal injection:

- Intradermal injection was done only under anesthesia.
- The hair on the back was clipped and prepared with alcohol swab.
- The needle was inserted in between the layers of skin on back at 30 degree.
- The syringe was aspirated to ensure proper placement
- Any sign of blood or other fluid indicated improper placement
- The drug was administered slowly with a maximum volume of 100µl for injection site to avoid tissue trauma. Successful injection resulted in small circular melt.

Oral feeding / Gavaging in mouse:

- Biomedical needles are used with 1-3ml syringe

- The distance from tip of nose to first rib was measured.The needle of measured length was used
- The syringe was filled with appropriate amount of drug.
- Mouse was restrained.
- The tip was slide down back of mouth moving lip forward in one felt motion
- Any substance felt indicated improper placement
- The drug was administered once the needle was properly placed.

STUDY OF EFFECT OF HEPATIC MICROSOMAL ENZYME INDUCERS ON THE PHENOBARBITONE SLEEPING TIME IN MICE

AIM: To study the effect of hepatic microsomal enzyme induction on the duration of action of pheno-barbitone.

REQUIREMENTS: Animal-Mice

Drugs- Phenobarbitone sodium (dose: 50mg/kg IP), Pentobarbital sodium (dose: 45 mg/kg IP)

PRINCIPLE: The drugs which induce hepatic microsomal oxidative enzyme system enhance the metabolism of other drugs. As a result, in the presence of an enzyme-inducer the duration action of second drug will be reduced. This has significant clinical relevance because when more than one drug is administered at a time one drug may modify the action of another through the microsomal enzyme inducing property.

The common drugs which induce hepatic microsomal enzyme system are phenobarbitone and meprobamate. Co-administration of any drug with either of these drugs may affect the disposition of the second drug and therefore, the desired pharmacological effects.

PROCEDURE:

1. Weigh and number the animals. Divide them into two groups, each comprising of at least 6 mice.
2. To the first group inject phenobarbitone once daily for 5 days. To get the second group inject distilled water, similarly for 5days.
3. One hour after the last dose of the phenobarbitone on the 5th day, inject phenobarbital to the both groups.
4. Note the onset and duration of sleep due to phenobarbitone in both the groups.

INFERENCE: Animals pretreated with phenobarbitone sleep for shorter duration of time as compared to animals treated with distilled water.

REPORT: Effect of hepatic microsomal inducers on phenobarbitone sleeping time in mice was studied.

EFFECT OF DRUGS ON THE CILIARY MOTILITY OF FROG OESOPHAGUS

AIM: To find out the effects of drugs on the ciliary motility of frog esophagus using Expharm T2 version.

PRINCIPLE:

Frog oesophagus contains cilia. Ciliary motility depends on action of acetylcholine in mucous membrane. ACh causes contraction of cilia leading to increased movements. Cholinergic drugs produce similar effect while anti-cholinergics paralyze cilia and decrease their movements. This experiment deals with a few such drugs to demonstrate their effect.

REQUIREMENTS:

1. Frog
2. Poppy seeds
3. Frog board
4. Stop watch

DRUG & SOLUTIONS

a) Acetylcholine 10%
b) Physostigmine 10%
c) Atropine 0.1%
d) Frog Ringer

Set up


A poppy seed being placed on the oesophagus


Poppy seeds

A frog is pithed and lower jaw is removed. The esophagus is slit open from buccal cavity to the stomach and everted to fix it on a wooden board with pins. Blood is wiped away by a cotton swab dipped in frog Ringer solution. The surface is moistened with frog Ringer. A poppy seed is placed at the cephalic end and its movements and time taken to travel a fixed distance on the esophagus are observed

PROCEDURE:

I. Determine the distance of seed movement. The starting point and the end point will be pins fixed at the cephalic end and caudal (distal) end respectively.
II. Instill frog-Ringer on the surface of the oesophagus. Place a poppy seed at the cephalic end of oesophagus. The seed starts moving due to ciliary motility. When the seed crosses the starting point (cephalic end pins), start the stop watch / clock. Stop it when the seed reaches the distal pins.
III. Note down the time taken for the seed to travel the distance. Repeat step 2 to get three readings.
IV. Instill ACh and take three readings.
V. Repeat step 2 and 3.
VI. Instill physostigmine and take three readings.
VII. Repeat step 2 and 3.
VIII. Instill atropine and take three readings.
IX. Instill ACh (without using frog-Ringer after step 6) and observe its effect. Compare it with the effect obtained with ACh alone (step 4).
X. Tabulate your readings and write conclusions.

Note:

- Test each drug including Ringer thrice and calculate average reading for each drug.

- Readings with Ringer are taken as control and compared with test (drug) readings.
- Take separate control readings for each drug i.e. Before testing any drug, take readings with Ringer.
- Use new preparations (frog) for each drug. To observe interactions, drugs should be applied on the same preparation (frog) consecutively without using Ringer in between them.

Observation:

Effect of drugs on the ciliary motility of frog oesophagus

S.NO.	Drugs	Reading 1	Reading 2	Reading 3	Mean
1	Ringer				
2	Acetycholine				
3	Physostimine				
4	Atropine				

Report:

EFFECT OF DRUGS ON RABBIT EYE

AIM: To study the effects of drugs on rabbit eye.

PRINCIPLE: Local action of large number of drugs in an eye can be achieved without systemic effect by application of drugs belonging to antimicrobial, autonomic or local anesthetic groups, the eye is supplied with both sympathetic and parasympathetic nerves.Ciliary muscles are supplied by parasympathetic nerves, when it contracts the ciliary body is mooned inwards and forwards because of the lens bulges forward and the eye is accommodated for near vision. The opposite effect is produced by the relaxation of ciliary muscles resulting in paralysis of accomidation.

Topically applied drugs can effect the eye by changing conjuctival conjuction, papillary size, light reflux, corneal sensitivity, and intraocular pressure. The pupillary size can be measured by placing a transparent plastic scale in front of eyes as close as possible.

Atropine an anti-n=muscarinic agents blocks the effects of endogenously released acetylcholine on the circular muscles of the iris and muscles f ciliary produces mydriasis and spasm of accomidngation leading to cycloplegia but without producing loss of corneal reflux.

REQUIREMENTS:

Animals- rabbit (2 - 5kgs)

Drugs- Atropine (1.1%w/v)

Equipments- rabbit holder, torch light

PROCEDURE:

- Rabbits were placed in the rabbit holder, the heads kept outside.
- The pupil size was observed in both the eyes
- The effect of light reflux were examined by holding the torch near to the eye and moving the light beam to and for both the animals

- The corneal reflex was examined by touching a side of cornea with a cotton swab or tip
- Few drops of atropine solution were instilled in the conjunctiva (4-6 times) over a period of 8-10 minutes in the right eye of the rabbit.
- The left eye was served as control, saline was instilled.
- The pupillary size, light reflux, corneal reflex action was recorded after 10 min after drug instillation.
- Observations were tabulated for a period of 60 minutes.

S.No.	Time (Min)	Pupil Size (mm)		Light reflex		Corneal reflex	
		Control (Left eye)	Test (Right eye)	Control (Left eye)	Test (Right eye)	Control (Left eye)	Test (Right eye)
1	0						
2	5						
3	10						
4	15						
5	20						

+ Response - No response

REPORT:

The effects of drug on rabbit eye was observed.

EFFECT OF SKELETAL MUSCLE RELAXANTS USING ROTA-ROD APPARATUS

AIM: To study the effect of CNS suppressant and muscle relaxant drug on mice using rotarod apparatus

INTRODUCTION: Rota-rod apparatus has a horizontal grooved rod rotating at a fixed speed, the mice are made to balance on this rod. Dependent upon their motor co-ordination. Central nervous activity and grip strength the animal either stay on the rotating rod for specific time and after that fall down on platform. The floor of each compartment has sensor, that deactivates the tissue and the exact fall off time for each rat is displayed on respective display.

PRINCIPLE: Reduction of motor co-ordination CNS depression and skeletal muscle relaxation leads to decrease in the fall off time and less no. of free riding indicates that administered drug has CNS depressant or muscle relaxant activity that either lead to decrease in the motor co-ordination or decrease in the gripping power.

PROCEDURE:

Animals are divided into 2 groups

- Administer one group with the drug to be tested and other with vehicle by intraperitoneal route.
- Click on cotton
- When the animal falls off respectively time will be displayed on the timer

CONCLUSION:

The observed reduction in the full-off time and free-riding shows that Diazepam at 1mg/kg I.P dose decrease in motor co-ordination and decrease in muscle strength.

REPORT:

CNS suppressant and skeletal muscle relaxant was evaluated by suing Ex-pharma software (rotarod apparatus).

EFFECT OF DRUGS ON LOCOMOTOR ACTIVITY USING ACTOPHOTOMETER

AIM: To evaluate the effect of diazepam on locomotor activity of mice using Actophotometer

INTRODUCTION: (PRINCIPLE)

The drug acting on CNS as stimulant or suppressants effect the locomotor activity in experimental animals. Such changes in the motor activity can be easily determined by using simple techniques in which the animal treated with the drugs is kept in an open compartment having square making on its floor. The lines of such squares crossed by the animals are counted by the observer. The number of lines crossed correlates with the locomotor activity. Alternatively, the locomotor activity ca be determined using Actophotometer or more sensitively by using automated video tracking system in addiction to the horizontal movement the vertical movements is also considered as a parameter associated with locomotor activity.

EQUIPMENT:

Actophotometer has a central chamber with arrangement of light sources and photocells at the base of two opposite walls. The light of each source is focused on a photocell. Interruption in the path of light the photocells and this is counted as a measure of horizontal chamber activity of the mice kept in the chamber.

DRUGS:

1. Control- Vehicle
2. Test- Diazepam

PROCEDURE:

1. Animals are divided into two groups (6 animals in each)
2. Administer one group with the drug to be tested and other with the vehicle by oral route
3. Put one animal at a time in the Actophotometer

4. Start the instrument
5. Count the locomotor activity for 10 minutes
6. Repeat the procedure at the interval of 30 minutes
7. Record the observation
8. The micein test group are injected Diazepam and locomotor activity is measured twice as started above

CONCLUSION:

The observed reduction in locomotor index shows that the given test drug (DIAZEPEM) exerts inhibition of the locomotor activity

REPORT:

The locomotor activity of diazepam was evaluated by using Actophotometer(EX-Pharma software).

ANTICONVULSANT EFFECT OF DRUGS BY MES AND PTZ METHOD

AIM: To study the anti-convulsant activity of phenytoin against maximal electro-shock-induced convulsions in rats

REQUIRMENTS: Animal-Rat

Drugs- phenytoin

Equipment: Electro-convulsometer, corneal electrode, stop watch.

PRINCIPLE:

Different type of epilepsies, i.e., grand mal, petit mal or psychomotor type, can be studied under in laboratory animals. The maximal electro-shock (MES) - induced convulsions in animal represents grand mal type of epilepsy. Similarly, chemo-convulsions due to pentylenetetrazol which produce clonic-type of convulsions resemble petit mal type of convulsions in man. These are the two types procedures used to study the convulsions, and to test anti-convulsant drugs in laboratory animal.

In MES- convulsions electro shock is applied through the corneal electrodes. Through optic stimulation cortical excitation is produced. The MES-convulsions are divided into 5 phases such as

(a). Tonic flexion

(b). Tonic Extensor

(c) clonic convulsions

(d) stupor and

(e) recovery or death.

A substance is known to possess anti-convulsant property if it reduces or abolishes the extensor phase of MES-convulsions. This procedure may be used to study convulsions

both in rats and mice. It is advised that the students should have complete background of the pharmacology of anti-epileptic drugs before performing the experiment.

PROCEDURE:

1.Weigh and number the animals. Divide them into two groups each consisting 4-5 rats. One group is used as control and the other for drug treatment.

2. Hold the animal properly, place corneal electrodes on the cornea and apply the prescribed current. Note different stages of conclusions i.e., (a). Tonic flexion (b). Tonic extensor phase (c). Chlonic convulsions (d) stupor and (e). recovery or death. Note the time (sec) spent by the animal in each phase of the convulsions. Repeat with other animals of control group.

3. Inject phenytoin intraperitonially to a group of 4-5 rats. Wait for the 30 min and subject the animals to electro convulsions have described in step2.

4. Note the reduction in time or abolition of tonic extensor phase of MES-convulsions.

INFERENCE: A reduction or complete abolition of tonic- extensor phase is considered anticonvulsant property of the drug. Phenytoin abolishes MES-induced tonic-extensors.

REPORT: The effect of anticonvulsant drug by MES and PTZ method are seen

STUDY OF ANXIOLYTIC ACTIVITY OF DRUGS USING MICE/RATS

AIM: To study anxiolytic effect of diazepam in mice using elevated zero-maze

REQUIREMENTS: Animal-Mice

Drugs-Diazepam

Equipment: Zero-maze

PRINCIPLE: Anxiety is defined as a feeling of apprehension, uncertainty and tension stemming from the anticipation of an imagined or unreal threat. Elevated zero-maze test is a behavioral test of anxiety based on naturalistic tendency of rodents to avoid open and elevated areas. It is similar to the more widely used elevated plus maze open and closed arms are arranged circularly, eliminating the central area which removes ambiguity in interpretation of time spend ion the central square of the traditional design. The maze is an elevated (40 CENTIMETERS), white or black, annular having outer diameter of 45 cm and inner diameter 30cm. The runway ring where the mouse can explore is of 6cm width, which is divided into four quadratants, to opposing "open" quadrants without walls and to opposing "closed" quadrants having 12cm high walls. Open quadrants have the ridge of 2-3mm to prevent the mouse to fall off the walls have thickness of 0.75cm. The model is widely used for evaluation of anxiolytic agents belonging to chemically different classes of drugs.

PROCEDURE:

1. Weigh and number the animals. And divide them into two groups each consist the minimum of 6 mice. One group is used as control and other for drug treatment.
2. Place the animal individually in the open arm facing towards to closed arm. Start the stop watch and note the following parameters for the period of 6minutes
   (a). Latency to enter to open arm
   (b). average time each animal spends in the open/ closed arm.
   (c). total number of entries in the open arm
   (d). stretching's
3. Clean the maze to using the tissue paper after each trial.

4. Inject the diazepam to the test group after 30minutes place the animal individually as described above and note all the parameters as in step 2

5. Compare the time spent in open arm, latency to open arm number of entries in open arm as well as number of stretching's.

REPORT: Effect of anxiolytic activity of drugs is studied in mice/rats.

STUDY OF LOCAL ANAESTHETICS BY DIFFERENT METHODS

AIM: To study the local anesthetic property by different methods.

REQUIREMENTS:

Animal-frog

Drug-Procaine hydrochloride stock solution (1%w/v), hydrochloric acid (0.1N)

Equipment: Frog board and surgical instruments

PRINCIPLE: Local anesthetics reversibly block impulse conduction along nerve axons and excitable membranes. Their action is used to produce local or regional anesthesia and for blocking pain sensation. The local anesthetic property can be easily studied by using any of the following methods, i.e., (a) Nerve block anesthesia(Solman method) where the drug is applied close to nerve trunk; (b)Surface anesthesia (Administered the drug into conjunctiva of the eye and study corneal reflux to pointed object); or (c) Infiltration anesthesia(where the drug is administered intradermally and the injection site is tested for reaction to pin pricks.

PROCEDURE:

1. Decerebrate the frog 100 to 150 grams and destroy the upper part of the spinal cord with the help of pithing needle.
2. Cut open the abdomen and remove all the abdominal organs, so that a pouch is made of abdominal walls.
3. Expose the spinal nerves in the cavity.
4. Put the frog on the frog board with two of its hind legs hanging free from the board. Alternatively, the frog may be pinned to the board placed vertically so that its hindlegs hang free.
5. Immerse the right hindleg in the beaker containing 0.1N HCl. Note brisk reflex withdrawal of the leg.
6. Wash the immersed leg with flowing water or by dipping the leg in the beaker containing normal saline.
7. Repeat the same with another leg.

8. Place about 10ml of 1%w/v procaine hydrochloride in the abdominal pouch. The sciatic nerve flex is exposed to the local anesthetic actions of the drug. Allow the drug to act for 5 minutes.
9. Immerse right and left hind legs in succession in the beaker containing acid as before.
10. Note the delay in the reflex withdrawal of legs.

INFERENCE: Direct application of procaine hydrochloride to the nerve trunk produces local anesthesia as foot withdrawal reflexes are blocked in the frog.

REPORT: The action of local anesthetics was studied by different methods.