MIPER

MOTHER THERESA INSTITUTE OF PHARMACEUTICAL EDUCATION & RESEARCH

miperknlapindia@gmail.com

IV SEMESTER (II-B.PHARM)

PHARMACOLOGY - I

PRACTICAL LAB MANUAL

CONTENT

	INTRODUCTION TO EXPERIMENTAL PHARMACOLOGY	
1.	**COMMONLY USED INSTRUMENTS IN EXPERIMENTAL PHARMACOLOGY**	
2.	**STUDY OF COMMON LABORATORY ANIMALS**	
3.	**CPCSEA GUIDELINES FOR LABORATORY ANIMAL FACILITY.**	
4.	**STUDY OF DIFFRENT ROUTE OF DRUG ADMINISTRATION**	
5.	**EFFECT OF DRUGS ON RABBIT EYE**	
6.	**EFFECT OF DRUGS ON CILIARY MOTILITY OF FROG OESOPHAGUS**	
7.	**EFFECTS OF SKELTAL MUSCLE RELAXANT USING ROTA –ROD APPARATUS**	
8.	**STUDY OF DRUGS ON LOCOMOTOR ACTIVITY USING ACTOPHOTOMETER**	
9.	**ANTICONVULSANT EFFECT OF DRUGS BY MAXIMAL ELECTRO-SHOCK INDUCED CONVULSIONS IN RATS**	
10.	**STUDY THE EFFECT OF HEPATIC MICROSOMAL ENZYME INDUCTION**	
11.	**STUDY OF STEREOTYPE ANTI CATATONIC ACTIVITY OF DRUGS ON RAT/MICE**	
12.	**STUDY OF ANXIOLYTIC OF DRUGS USING MICE/RAT**	
13.	**STUDY OF LOCAL ANAESTHETIC PROPERTY BY FOOT WITHDRAWAL METHOD**	

INTRODUCTION TO EXPERIMENTAL PHARMACOLOGY

Pharmacology is the science which deals with the study of drugs. the word 'PHARMACOLOGY' is derived from the Greek words '*Pharmakon'* (a drug or poison) and *logos* (discourse). It broadly covers the information about the history, source, physiochemical properties, physiological 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 is relatively the youngest branch of basic medical sciences. The advancement in the field of electrophysiology, biochemistry, molecular biology and electronic or digital recording systems and software's have enriched and broadened the horizons of experimental pharmacology.

The main aims of the experimental pharmacology are to

1. Find out a therapeutic agent suitable for human use
2. Study the toxicity of a drug
3. Study the mechanism of action of drugs

   Since experimental pharmacology involves the discovery of new drugs or to study the action of existing drugs it is done in two main stages

   - Preclinical experimental pharmacology which involves the identification and optimization of novel chemical lead structures and testing them on animals and animal tissues or organs for their biological actions
   - Clinical pharmacology where testing of drugs is done on human volunteers and patients for assessing the pharmacokinetics, safety and efficacy in humans.

Ex .No: 01

COMMONLY USED INSTRUMENTS IN EXPERIMENTAL PHARMACOLOGY

In spite of a tremendous development in electronic devices and recording systems are followed in institutions and research laboratory. Some common instruments are used in experimental pharmacology

Organ bath:

The tissue bath used to put the animal tissue for studying the drug actions is called student organ bath. This was first designed by Rudolph Magnus in 1904. The organ bath essentially consist of

- An outer jacket made of up of steel or glass or Perspex.
- The inner organ or tissue bath made up of glass with a capacity varying from 10 -50 ml
- Thermostatically controlled heating rod
- Stirrer to keep the water in the outer jacket at uniform temperature
- Oxygen or delivery glass tube which also serves as tissue holder
- Glass coil, one end of which is connected having the physiological salt solution.

  The student organ bath having two units of inner tissue bath is called double unit organ bath.

Rota rod apparatus:

For the study of muscle relaxant property of diazepam in mice. The loss of muscle grib in an indicator of muscle relaxation. This effect can be studied in animals using an inclined plane or rotating rods.

Actophotometer:

It is used to study CNS depressant property of chlorpromazine on the loco motor activity of mice. CNS depressant drugs like alcohol reduces the motor activity with the stimulants like caffeine and amphetamines increases the activity. The actophotometer operates on photoelectric cell which is connected in circuit with counter.

Electro-convulsiometer

It is used to study the anticonvulsant activity of phenytoin against electro-convusiometer induced in rat. The electric shock is applied through the corneal electrodes; it produces 5 phases such as tonic flexion, tonic extensor, clonic convulsion, stupor, and recovery/death.

Pole climbing apparatus

It is used to study the anxiolytic activity in rat and mice. The basic principles of pole climbing apparatus is based on a neuro-chemical mechanism of anxiety disorders drugs like benzodiazepam are used.

Analgesiometer:

It is used to study the analgesic effects of drugs in mice and rat. The inducer of pain such as heat, physio compression and chemical inducers. The basal reaction time was noted as a inference of pain sensation drugs like morphine and other NSAID are used.

Metabolic cage:

It is used to study the metabolic parameters such as faecal an urine for the study of purgative or laxative, animals such as rat and mice are used.

Ex .No: 02

STUDY OF COMMON LABORATORY ANIMALS

1. **Guinea pig**

Guinea pigs (400-600g) are the commonly used experimental animals. they are very docile and easy to raise and maintain they are highly sensitive to histamine. They are used in experimental asthma to study bronchodilators . they are also used to local anaesthetics and as a model in amoebiasis and cholera as they are sensitive to this micro organism.

2. **Albino rat**

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 and porton.

3. **Albino mouse**

White mice are the smallest laboratory animals used. Mice are also easy to breed and maintain. They are small in size (25-30g) and therefore, easy to breed and maintain. They are sensitive to most of the drugs used in experimental pharmacology. Mice are used extensively in toxicity study, bio assay of insulin, testing of analgesics, CNS active drugs and chemotherapeutic agents. More recently mouse brain as well as primary cell culture of mouse spinal cord neurons are used in neuro pharmacology for studying neurotransmitters receptor functions . the other strains of mice used are laca and balb/C.

4. **Rabbit**

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.

5. **Frog**

Frogs (150-200g) were one time extensively used in experimental pharmacology. However, recently the use of wild frogs for experimental purposes has been banned. Earlier, frogs were used for isolated heart, rectus abdominis muscle preparation, study of muscle nerve and ciliary movements, respectively. Frogs were also used for the study of nerve block type of local anaesthetics . frogs are inexpensive and easily available, and the ban on the use of frogs has been debated.

Other animals

Cats, dogs and monkeys are used for pharmacological investigations of drugs. Cats and dogs were one time 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 (USFDA) for preclinical testing of new drugs.

Alternatives to animal experimentation

Because of the growing concern on the use of animals in biomedical research in several countries have passed legislation to prevent or usage of animal experimentation . these include experiment with tissue and body fluids of normal animals and human use of micro organisms, primary cell culture and cell lines, use of models and computer simulation and software are being used now a days

As per the common laboratory alternate animals

Zebra fish	***Danio rerio***	Developmental assay Drug discovery Neurobiology Toxicity
Worms	***Habolitis***	Developmental assay Neurobiology Toxicity
Fruit fly	***Drosphila***	Cancer biology Developmental assay Neuroscience
Tunicates	***Ciona sp***	Developmental assay Neuroscience
Star fish	***Echinoderms***	Developmental assay neuroscience

Ex .No: 03

CPCSEA GUIDELINES FOR LABORATORY ANIMAL FACILITY.

Good Laboratory Practices (GLP) for animal facilities is intended to assure quality maintenance and welfare of animals used in laboratory studies while conducting biomedical and behavioural research and testing of products.

1. GOAL

The goal of these Guidelines is to promote the humane care of animals used in biomedical and behavioural research and testing with the basic objective of providing specifications that will enhance animal well being, quality in the pursuit of advancement of biological knowledge that is relevant to humans and animals.

2. VETERINARY CARE

a. Adequate veterinary care must be provided and is the responsibility of a veterinarian or a person who has training or experience in laboratory animal sciences and medicine.

b. Daily observation of animals can be accomplished by someone other than a veterinarian; however, a mechanism of direct and frequent communication should be adopted so that timely and accurate information on problems in animal health, behaviour, and well being is conveyed to the attending veterinarian.

c. The veterinarian can also help the establishment in designing appropriate policies and procedures for ancillary aspects of veterinary care, such as use of appropriate methods to prevent and control diseases (e.g. vaccination and other prophylaxis, disease monitoring and surveillance, quarantine and isolation), operative and post-operative care, diagnosis and treatment of diseases as well as injuries. reviewing protocols and proposals, animal husbandry and animal welfare; monitoring occupational health hazards containment, and zoonosis control programs; and supervising animal nutrition and sanitation. Institutional requirements will determine the need for full-time or part-time or consultative veterinary services.

3. ANIMAL PROCUREMENT

a. All animals (like cattle, buffalo, sheep, goat, pigs, equine etc.) must be acquired lawfully as per the CPCSEA guidelines. Small animals and dogs can be procured from registered breeders. Large animals can be procured from farm, farmers or as per guidance of wild life department, as is done in case of macaques. Cats can be bred for their use. Rodents can be

imported from abroad after necessary licence from Director General of Foreign trade (DGFT) is obtained for import.

b. A health surveillance program for screening incoming animals should be carried out before purchase to assess animal quality. Methods of transportation should also be taken into account

c. Each consignment of animals should be inspected for compliance with procurement specifications, and the animals should be quarantined and stabilized according to procedures appropriate for the species and circumstances.

4. QUARANTINE, STABILIZATION AND SEPARATION

a. Quarantine is the separation of newly received animals from those already in the facility until the health and possibly the microbial status of the newly received animals have been determined. An effective quarantine minimizes the chance for introduction of pathogens into an established colony. The duration at quarantine in small lab animals is from one week to one month and large animals allowed up to 6 weeks (cat, dog, monkey, etc). However, duration of quarantine can be increased depending on type of infection / suspected infection noticed in the animals.

b. Effective quarantine procedures should be used for non-human primates to help limit exposure of humans to zoonotic infections. The period varies from 2 to 3 months depending on the reaction of TB testing. Any macaque found positive for TB for at least two times and shows signs of weight loss or ill health should be euthanized as is practiced internationally to prevent spreading of TB to workers and other macaques.

c. Regardless of the duration of quarantine, newly received animals should be given a period for physiologic, psychologic and nutritional stabilization before their use. The length of time stabilization will depend on the type and duration of animal transportation, the species involved and the intended use of the animals.

d. Physical separation of animals by species is recommended to prevent interspecies disease transmission and to eliminate anxiety and possible physiological and behavioural changes due to interspecies conflict.

e. Such separation is usually accomplished by housing different species in separate rooms; however, cubicles, laminar-flow units, cages that have filtered air or separate ventilation, and isolators can be used as suitable alternatives.

f. In some instances, it shall be acceptable to house different species in the same room, for example, if two species have a similar pathogenic status and are behaviourally compatible.

Separate set of personnel should be identified for taking care of these infected (sick) animals and other workers should be restricted from entering in to the facilities unless otherwise required and after handling these animals they should not be handling any other animals in the facilities

5. SURVEILLANCE, DIAGNOSIS, TREATMENT AND CONTROL OF DISEASE

(a) All animals should be observed for signs of illness, injury, or abnormal behaviour by animal house staff. As a rule, this should occur daily, but more-frequent observations might be warranted, such as during postoperative recovery or when animals are ill or have a physical deficit. It is imperative that appropriate methods be in place for disease surveillance and diagnosis

(b) Post-mortem examination and signs of illness, distress, or other deviations from normal health condition in animals should be reported promptly to ensure appropriate and timely delivery of veterinary medical care. Animals that show signs of a contagious disease should be isolated from healthy animals in the colony. If an entire room of animals is known or believed to be exposed to an infectious agent (e.g. Mycobacterium tuberculosis in non-human primates), the group should be kept intact and isolated during the process of diagnosis, treatment, and control. Animals suffering from contagious diseases like Tuberculosis etc. must be euthanized as is practiced internationally to prevent its spread to other animals and often animal handlers.

(c) The isolation, quarantine and stabilization programs for newly arrived animals are necessary to provide time to assess their health status, allow them to recover from the stress of shipment and an opportunity to adapt to their new environment. The extent of these programs depends on several factors, including species and source of the animals as well as their intended use. For some animals, such as rodents obtained from reliable sources for which health status is known, visual inspection on arrival may suffice. For species such as nonhuman primates, farm animals, wild animals, dogs, cats and non-specific pathogen free rabbits and rodents, appropriate quarantine and isolation procedures must be employed.

(d) Preventive medicine programs such as vaccinations, ecto- and endoparasite treatments and other disease control measures should be initiated according to currently acceptable veterinary medical practices appropriate to the particular species and source. Only animals of defined health status should be used in research and testing unless a specific, naturally occurring or

induced disease state is being studied. Systems should be established to protect animals within the institution from exposure to diseases.

(e) Transgenic and mutant animals may be particularly susceptible to diseases and may require special protection to ensure their health. Systems to prevent spread of disease may include facility design features, containment/isolation equipment, and use of standard operating procedures. Training of animal care and research staff is essential to prevent spread of animal diseases.

(f) Disease surveillance is a major responsibility of the veterinarian and should include routine monitoring of colony animals for the presence of parasitic and microbiological agents that may cause overt or unapparent disease. Additionally, cells, tissues, fluids, and transplantable tumors that are to be used in animals should be monitored for infectious or parasitic agents that may cause disease in animals. The type and intensity of monitoring necessary will depend upon professional veterinary judgment and the species, source, use and number of animals housed and used in the facility.

(g) Diagnostic laboratory services must be available and used as appropriate. Laboratory services should include necropsy, histopathology, microbiology, clinical pathology, serology, and parasitology as well as other routine or specialized laboratory procedures, as needed. It is not necessary that all of these services be available within the animal facility (Facilities from other laboratories with appropriate capabilities may be used).

(h) Animals with infectious / contagious disease must be isolated from others by placin them in isolation units or separate rooms appropriate for the containment of the agents of concern. In certain circumstances, when an entire group of animals is known or suspected to be exposed or infected, it may be appropriate to keep the group intact during the time necessary for diagnosis and treatment, for taking other control measures, or for completion of a project.

(i) The veterinarian must have authority to use appropriate treatment or control measures, including euthanasia in consultation with at least one more additional veterinarian if required, following diagnosis of an animal disease or injury. If possible, the veterinarian should discuss the situation with the principal investigator to determine a course of action consistent with experimental goals. However, if the principal investigator is not available, or if agreement cannot be reached, the veterinarian must have authority to act to protect the health and well- being of the institutional animal colony and workers.

6. ANIMAL CARE AND TECHNICAL PERSONNEL

(a) Animal care programs require technical and husbandry support. Institutions should employ people trained in laboratory animal science or provide for both formal and on-the-job training to ensure effective implementation of the program (Annexure-7).

7. PERSONAL HYGIENE

(a) It is essential that the animal care staff maintain a high standard of personal cleanliness. Facilities and supplies for meeting this obligation should be provided with appropriate Personnel Protective Equipment (PPE) e.g. showers, change of uniforms, footwear etc.

(b) Clothing suitable for use in the animal facility should be supplied and laundered by the institution. A commercial laundering service is acceptable in many situations; however, institutional facilities should be used to decontaminate clothing exposed to potentially hazardous microbial agents or toxic substances. It is acceptable to use disposable gloves, masks, head covers, coats, coveralls and shoe covers. Personnel should change clothing as often as is necessary to maintain personal hygiene. Outer garments worn in the animal rooms should not be worn outside the animal facility.

(c) Washing and showering facilities appropriate to the program should be available. Personnel should not be permitted to eat, drink, smoke or apply cosmetics and perfumes in animal rooms. They should finish the work with animals as early as possible and sit somewhere else, outside and not in the animal rooms / areas. A separate area or room should be made available for these purposes.

8. ANIMAL EXPERIMENTATION INVOLVING HAZARDOUS AGENTS

(a) Institutions should have policies governing experimentation with hazardous agents. Institutional Bio-safety Committee whose members are knowledgeable about hazardous agents are in place in most of the higher-level education, research institutes and in many pharmaceutical industries for taking care of safety issues. This committee shall also examine the proposal on animal experiments involving hazardous agents in addition to its existing functions

(b) Since the use of animals in such studies requires special considerations, the procedures and the facilities to be used must be reviewed by both the Institutional Bio-safety committee and Institutional Animal Ethics Committee (IAEC). Disposing of tissues and fluids from such used animals must also be appropriately governed as per the laid in practices of the institution / bio-safety regulation.

9. MULTIPLE SURGICAL PROCEDURES ON SINGLE ANIMAL

(a) Multiple surgical procedures on a single animal for any testing or experiment are not to be practiced unless specified in a protocol only approved by the IAEC.

(b) Individual animals should not be used in more than one experiment, either in the same or different projects, without the express approval of the IAEC. However, it is noted that Appropriate re-use of animals may reduce the total number of animals used in a project, result in better design of experiments, and reduce stress or avoid pain to additional animals. Animals that are used in more than one experiment should be permitted to recover fully from the first experiment before the subsequent experiment is performed. Certification of attending veterinarian is however, required before subjecting animal to the second experiment.

10. DURATIONS OF EXPERIMENTS

No animal should be used for experimentation for more than 3 years unless adequate justification is provided.

11. PHYSICAL RESTRAINT

(a) Brief physical restraint of animals for examination, collection of samples, and a variety of other clinical and experimental manipulations can be accomplished manually or with devices be suitable in size and design for the animal being held and operated properly to minimize stress and avoid injury to the animal.

(b) Prolonged restraint of any animal, including the chairing of non-human primates, should be avoided unless essential to the research objectives. Less restrictive systems, such as the tether system or the pole and collar system should be used when compatible with research objectives.

(c) Following points should be considered during handling and restraining animals:-

i. Animals should be handled by competent individuals trained in methods that cause minimaldistress and injury (for example, a person with a publication to his/her credit and post/experience in relevant techniques for handling animals is preferable).

ii. The use of restraint devices is sometimes essential for the welfare of the animal and safety of the handler. Restraint devices should be used to the minimum

iii. extent, for the minimum period required to accomplish the purpose of the experiment and be appropriate for the animal.

iv. Tranquilisers or anaesthetics may initially be used to aid restraint but they may prolong recovery from the procedure. When these agents have been used, recovery of the animals

should be closely monitored.

(d) The following are important guidelines for the use of restraint equipments:

i. Restraint devices cannot be used simply as a convenience in handling or managing animals. The period of restraint should be the minimum required to accomplish the research objectives. Animals to be placed in restraint devices should be given training to adapt to the equipment, prior to initiation of the experimentation.

ii. Provision should be made for observation of the animal at appropriate intervals. Veterinary care should be provided if symptoms or illness associated with restraint are observed. The presence of illness, or severe behavioural change should be dealt with by temporary or permanent removal of the animal from restraint related protocol.

12. LOCATION OF ANIMAL FACILITIES TO LABORATORIES

Good animal husbandry and human comfort and health protection require physical separation of animal facilities from personnel areas such as offices, break room, training and education room.

 Laboratory animals are very sensitive to their living conditions. It is important that they shall be housed in an isolated building located as far away from human habitations as possible and not exposed to dust, smoke, noise, wild rodents, insects and birds. The building, cages and environment of animal rooms are the major factors, which affect the quality of animals.

 This separation can be accomplished by having the animal quarters in a separate building, wing, floor, or room. Careful planning should make it possible to place animal housing areas adjacent to or near laboratories, but separated from them by barriers such as entry locks, corridors, or floors.

 While planning an animal facility the space should be well divided for various activities. The animal rooms should occupy about 50-60% of the total constructed area and the remaining area should be utilized for services such as stores, washing, office and staff, machine rooms, quarantine and corridors. The environment of animal room (Macro-Environment) and animal cage (Microenvironment) are factors on which the production and experimental efficiency of the animal depends. Since animals are very sensitive to environmental changes, sharp fluctuations in temperature, humidity, light, sound and ventilation should be avoided. The recommended space requirements for animal rooms, for different species

13. FUNCTIONAL AREAS

(a) The size and nature of a facility will determine whether areas for separate service functions are

possible or necessary. Sufficient animal area required to:

- Ensure separation of species or isolation of individual projects when necessary;
- Receive, quarantine, and isolate animals; and
- Provide for animal housing.

(b) In facilities that are small, maintain few animals or maintain animals under special conditions (e.g., facilities exclusively used for housing germfree colonies or animals in runs and pens)some functional areas listed below could be unnecessary or included in a multipurpose area. Professional judgement must be exercised when developing a practical system for animal care.

- Specialized laboratories or
- Individual areas contiguous with or near animal housing areas for such activities as surgery,intensive care, necropsy, radiography, preparation of special diets, experimental manipulation,treatment, and diagnostic laboratory procedures containment facilities or
- Equipment, if hazardous biological, physical, or chemical agents are to be used
- Receiving and storage areas for food, bedding
- Pharmaceuticals and biologics, and supplies
- Space for administration, supervision, and direction of the facility
- Showers, sinks, lockers and toilets for personnel
- An area for washing and sterilization equipment and supplies,
- An autoclave for equipment
- Food, and bedding; and separate areas
- For holding soiled and cleaned equipment
- An area for repairing cages and equipment
- An area to store wastes prior to incineration or removal

14. PHYSICAL FACILITIES

The physical condition and design of animal facility determine, to a great extent, the efficiency and economy of this operation. The design and size of an animal facility depend on the scope of institutional research activities, animals to be housed, physical relationship to the rest of the institution, and geographic location. A well planned, properly maintained facility is an important element in good animal care.

(a) Housing facility should be compatible with the needs of the species to be housed.

(b) Housing Facilities should be designed and operated to facilitate control of environmental factors to exclude vermin and limit contamination associated with the housing of animals,

delivery of food, water, bedding, and the entry of people and other animals.

(c) Housing Facilities should be maintained in good repair. Walls and floors should be constructedof durable materials with surfaces that can be cleaned and disinfected readily.

(d) Housing Facilities should be kept clean and tidy and operated to achieve maximum possiblehygiene.

(e) There should be a pest control programme to monitor and control vermin.

(f) There should be adequate and appropriate storage areas for food, bedding and equipment.

(g) Deodorants designed to mask animal odours should not be used in Housing Facilities as they may expose animals to volatile compounds which can alter metabolic processes. In addition, deodorants must not be used as a substitute for good cage and equipment cleaning practices and good ventilation.

(h) Cleaning practices should be monitored on a regular basis to ensure effective hygiene and sanitation. This may include visual inspection, monitoring water temperatures and microbiological testing of surfaces after cleaning.

(i) There should be proper water supply and drainage.

(j) There should be adequate contingency plans to cover such emergencies as flooding and fire, or the breakdown of lighting, heating, cooling or ventilation.

(k) In the interest of disease prevention and general animal welfare, access to the Housing Facilities by unauthorised persons should be restricted

Ex .No: 04

STUDY OF DIFFRENT ROUTE OF DRUG ADMINISTRATION

There are different route of drug administration by using laboratory animals

Aim:

1. To learn how to handle, treat preparing animals for experiments and ethical guidelines during the treatment.
2. Measure the required volume of drugs using aspectic techniques
3. To learn how to give a different types of route of drug administration

How to handle:

The good handling and restrained is most important techniques for correct administration. The restrain methods are 2 types

1. Physical method
2. Manual method
    - Double handled manual method
    - Single handled manual method

Route of drug administration

- Enternal route (oral and intregastric)
- Parentral (I.V, I.M,I.P,S.C,I.D)
- Topical route (surface of the skin membrane)

Enternal route:

The placement of drugs directly into any part of GIT directly it could be oral, sublingual, intra gastric.

Oral route:

The swallowing of a drug through mouth by the desired drug along with water (*ad libitum*) or food. It is economic and convenient , safe and some animals can be trained to cooperated voluntarily. This route is not referable since it is in accurate.

Intra gastric route:

The administration of fluid / drugs directly into oesophagus or stomach it is often used instead of mixing the substance in water or food. This method is accurate for the dosing od animals.

The gastric lavage tube is a small curved, metallic tube with the ball on the tip, it is often used without anaesthesia.

Parental route:

Subcutaneous route: the part of the subcutaneous injection and back of neck it doesn't required large volume of drugs suitable for suspension.

Intra peritoneal route:

Commonly used in rat and mice, rapid absorption due to large particle surface, mouse may injected compared with rat (ranges from 2-10ml/kg)

Intravenous route:

It is the most efficient route of drug administration. It should be used with the help of restraining devices in the appropriate size for the animals to be injected. The tail vein is suitable for drug administration.

Topical route:

Most convenient route for topical drugs, drugs like local anaesthetics, ointments and cream can be easily applied on the surface of the skin membrane. Eye drops and ointments were also used in this method. Animals like rabbit, guinea pig and rat are mainly use in this method.

Ex .No: 05

EFFECT OF DRUGS ON RABBIT EYE

Aim:

To study the Mydriatic effect of topically applied atropine on rabbit eye

Principle:

A large number of drugs are used for their local action in the eye as eye drops or eye ointments. Most of these drugs belongs to anti microbial, autonomic or local anaesthetic group. The eye is supplied by both sympathetic and parasympathetic nerves. The superior palpebral muscle and the dilator papillae of the iris have sympathetic supply. The sphincter pupillae of the iris has parasympathetic supply which exercises dominant control. The ciliary muscle is also supplied by parasympathetic nerve and when it contracts, the ciliary body is moved 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 muscle resulting in paralysis of accommodation. When the pupil dilates, the iris folds back near the opening of the canal of schlemn and the drainage of aqueous humour is decreased thereby increasing intraocular pressure. constriction of the pupil, by opposite action, will increase drainage and reduce intraocular pressure.

Topically applied drugs can affect the eye by changing conjuctival congestion, papillary size, light relex, corneal sensitivity and intraocular pressure. however the effects of drugs on papillary size, light reflex and corneal reflex can be easily studied by the students. the pupillary size can be measured by placing a transparent plastic scale in front of the eye but as close as possible. Light reflex is elicited by directing the light of a torch towards the pupil. The sensitivity of the cornea is tested by gently touching the cornea with a fine cotton swab stick from the side and not from the front of the eye. This elicits corneal reflex which manifests as blinking of the crystals.

Requirements:

Animal : Rabbit(2-5kg)

Drugs: Atropine (1%w/v) & Physostigmine (1%w/v)

Equipment: rabbit holder, pen torch

Procedure:

1. Place the rabbit in the rabbit holder by keeping the head outside
2. Observe size of the pupil in both the eyes
3. Examine the effect of light reflex by holding the torch in front of the eye moving light beam to and fro
4. Examine the corneal reflex by touching a side of the cornea with a cotton swab.
5. Instil a few drops of atropine solution in the conjunctiva over a period of 8-10 min in the right eye of the rabbit would serve as control. Instil normal saline in the left eye.
6. Record the pupillary size, light reflex and corneal refkex after ten minutes of drug initialisation and tabulate the observation.
7. Repeat the experiments with physostigmine and ephedrine

Observation

DRUG	**PUPILLARY SIZE**	**LIGHT REFLEX**	**CORNEAL REFLEX**
Saline	Normal	Present	Present
Physostigmine	Constriction	Present	Present
Ephedrine	Dilation	Present	Present
atropine	Dilation	Absent	Present

Ex .No: 06

EFFECT OF DRUGS ON CILIARY MOTILITY OF FROG OESOPHAGUS

Aim:

To study the effect of physostigmine and atropine on ciliary movement in frog buccal cavity.

Principles:

Cilia in the buccal cavity and in the oesophagus helps in the movement of food particles. Similarly, the importance of mucociliary function has been established in respiratory tract and of pulmonary diseases such as chronic bronchitis, asthma and in cystic fibrosis. The integrity of mucociliary function is very important in these air way diseases. Cilia exhibit a great degree of autonomy in that they are capable of functioning in the absence of nervous innervations. It has also been demonstrated that the Ach present in the mucous membrane of trachea and buccal cavity helps in the ciliary movement. Ach serves as a local hormone and the presence of choline acetylase support the fact that Ach is locally synthesised in the mucous membranes.

Requirements:

Animal: Frog

Drugs: Physostigmine stock solution (1µg/ml), Atropine stock solution (1µg/ml)

Physiological solution : Normal saline

Procedure:

1. Decapitate the frog and pin the frog to the frog board on its back.
2. Pin the lower jaw to the abdomen cutting sufficiently the buccal cavity and exposing the oesophagus wet by irrigating it with normal saline.
3. To assess the distance travelled by the particles, fix two points. One start from lower jaw to other beginning of oesophagus. Keep this distance constant to measure the time taken by the particle to move from a fixed point in the lower jaw to the beginning of the oesophagus.

4. Place a poppy seed or a small piece of cork at the premarked spot in jaw. Turn on the stop watch and note the time taken by the object to reach the beginning of the oesophagus . repeat this several times.
5. Put a few drops of physostigmine on the buccal cavity and after 10 min repeat step 4 note the tome.
6. Wash the buccal with normal saline. Put a few drops o atropine on the buccal cavity. After 10 min repeat the step 4. Note the time.
7. Find out the differences in the time taken by the object to move between the premarked distance in the buccal cavity in presence of saline, physostigmine and atropine.

Inference: Physostigmine reduces and atropine enhances the time taken by the object to move from the pre marked point in the lower jaw to reach the oesophagus , respectively .

Ex .No: 07

EFFECTS OF SKELTAL MUSCLE RELAXANT USING ROTA –ROD APPARATUS

Aim:

To study the muscle relaxant property of diazepam in mice using rota rod apparatus

Principle:

One of the important pharmacological action of anti anxiety agents of benzo diazepam class of drugs is muscle relaxaing property. The skeletal muscle relaxation together with taming or calming effect, these agents reduce anxiety and tension. The loss of muscle grip is an indication of muscle relaxation. This effect can be easily studied in animals using inclined plane or rotating rods.

The difference in the fall off time from the rotating rod between the control and diazepam treated animal is taken as an index of muscle relaxation . The angle of the slope of the inclined plane, or the rate of rotation of the rod should be adjusted such that a normal mouse can stay on the plane or on the rod for an appreciable period of time.

Requirement:

Animal : Mice (20-25gm)

Drugs: Diazepam of the drug and inject 1ml/100g of body weight of the mouse.

Equipment: Rota rod apparatus

Procedure:

1. Weigh the animals and number them.
2. Turn on the instrument select an appropriate speed (20-25rpm)
3. Place the animal one by one on the rotating rod. One can place more than one mouse. Note down the fall off time when the mouse falls from the rotating rod. A normal mouse generally falls off within 3-5 min
4. Inject diazepam to all animals. After 30 min repeat the experiments as done in step 3 . note the fall off time.
5. Compare the fall off time of animals before and after diazepam treatment.

Ex .No: 08

STUDY OF DRUGS ON LOCOMOTOR ACTIVITY USING ACTOPHOTOMETER

Aim:

To study the central nervous system depressant property of chlorpromazine on the locomotor activity of mice using actophotometer

Principle:

Most of the central nervous system acting drugs influencing the locomotor activities in man and animals. The CNS depressant drugs such as barbiturates and alcohol reduces the motor activity while the stimulants such as caffeine, amphetamines increases the activity. In other words , the locomotor activity can be index of wakefulness of mental activity. The locomotor activity can be easily measured using an actophotometer . which operates on photoelectric cells which are connected in circuit with a counter . when the beam of light falling on the photocell is cut off by the animal, a count is recorded. An actophotometer could have either circular or square arena in which the animal moves. Both rats and mice may be used for testing in this equipment.

Requirement:

Animal: Mice (20-25gm)

Drugs: Chlorpromazine hydrochloride

Equipment : Actophotometer

Procedure:

1. Weigh the animals and number them
2. Turn on the equipment and place individually each mouse in the activity cage for 10 min. Note the basal activity score of all the animals.
3. Inject chlorpromazine and after 30 min re –test each mouse for activity scores for 10 min. Note the difference in the activity before and after chlorpromazine.
4. Calculate percent decrease in motor activity .

Ex .No: 09

ANTICONVULSANT EFFECT OF DRUGS BY MAXIMAL ELECTRO-SHOCK INDUCED CONVULSIONS IN RATS

Aim:

To study the anti convulsant activity of phenytoin against maximal electro shock induced convulsion rats.

Principle:

Different types of epilepsy i.e., grant mal, petit mal or psychomotor type, can be studied in laboratory animals. The maximal electroshock (MES) induced convulsions in animals represent grandmal epilepsy. Similarly chemo- convulsion due to pentylene-tetrazol which produce clonic type of convulsion in man. These are the two procedures used to study convulsion, and to test anti convulsant drugs in laboratory animals.

In MES- convulsion electroshock is applied through the corneal electrodes. Through optic stimulation cortical excitation is produced. The MES convulsion are divided into five phases are

1. Tonic flexion
2. Tonic extensor
3. Clonic convulsion
4. Stupor
5. Recovery / death

A substance is known to posses anti convulsant property if it reduces or abolishes the extensor phase of MES convulsions both in rats and mice. It is advised the students should have complete background of the pharmacology of anti epileptics drugs before performing this experiment.

Requirement:

Animal : Rats (150-200 gm)

Drugs: phenytoin (dose 25 mg/kg); prepare a stock solution

Equipment : electro- convulsiometer, cornel electrode (150 mA current for 0.2 sec)

Stop watch.

Procedure:

1. Weigh and number the animals. Divide them into two groups each consisting of 4-5 rats. One group is used as control and the other for drugs treatment.
2. Hold the animals properly , place corneal electrodes on the cornea and apply the prescribed current. Note the difference stages of convulsion
3. Repeat with other animals of control group
4. Inject phenytoin I.P to group of 4-5 rats. Wait for 30 min and subjected to animals to electroconvulsions .
5. Note the reduction in time or abolition of tonic extensor phase of MES convulsions.

Ex .No: 10

STUDY THE EFFECT OF HEPATIC MICROSOMAL ENZYME INDUCTION

Aim:

To study the effect of hepatic microsomal enzyme induction on the duration of action of pentobarbital sodium.

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 of 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 effect the disposition of the second drug and therefore, the desired pharmacological effects. The students are advised to study various processes of drug metabolism before doing this experiment.

Requirement

Animal: Mice(20-25g)

Drugs: Phenobarbitone sodium (dose : 50mg\kg ip, once a day for 5 days. Prepare a stock containing 5mg\ml of the drug and inject 1ml\100g of body weight of animal. Prepare fresh solution every day). Pentobarbital sodium (dose45 mg\ml of the drug and inject 1 ml\100 g of body weight of animal).

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 the second group inject distilled water, similarly, for 5 days.

3. One hour after the last dose of phenobarbitone on the 5th day, inject pentobarbital to both the groups.
4. Note the onset and duration of sleepdue to pentobarbital in both the groups as done in the earlier experiment(5.1).

Inference the animals pretreated with phenbarbital sleep for shorter duration of the time as compared to animals treated with distilled water.

Ex .No: 11

STUDY OF STEREOTYPE ANTI CATATONIC ACTIVITY OF DRUGS ON RAT/MICE

Aim:

To study drug (phenothiazines)- induced catatonia (extrapyramidal side effect in rats.

2. to study the anticatatonic (antiparkinsonian) effect of scopolamine.

Principle:

Phenothiazine and butyrophenone type of antipsychotic drugs are known to produce extrapyramidal side-effects in man .these effects , such as akinesia, rigidity and tremors, are called parkinson's- like because in parkinson's disease the major clinical symptoms include difficulty to move and change posture (akinesia and rigidity) and tremors. These effects of antipsychotic drugs are due to excessive blockade of dopamine receptors in the extrapyramidal motor system. Therefore, phenothiazines (choloropromazine or perphenazine) are commonly used to produce parkinson's-like extrapyramidal symptoms in laboratory animals and to study anti-parkinsonism drugs.The students are advised to know the pharmacology of anti-parkinsonism drugs before performing this experiment.

Requirements

Animal: Rats (150-200 g)

Drugs: Perphenazine (dose : 5 mg/kg ip;prepare a stock solution containing 1 mg/ml of the drug and inject 0.5 ml/100g body weight of the animal). Scopolqmine (dose: 2 mg/kg, ip prepare a stock solution containing 0.4 mg/ml of the drug and inject 0.5 ml/100 g body weight of the animal)

Equipment two wooden blocks, one being 3 cm high and the other 9 cm high

Procedure

1. Weigh and number the animal. Divide the animals into two groups, one for control (for study of perphenazine effect) and the other for studying the effects of scopolamine. Each group should consist of at least 5 animals.

2. Inject perphenazine to control animals. observe severity of catatonic response (fig 5.14) as follows:
3. Observe the severity of catatonia at 5,15,30,45,60,90 and 120 min after perphenazine .
4. To the second group inject scopolamine and after 30 minute inject perphenazine to these animals already treated with scopolamine. Observe and score the severity of catatonic as in step 2.
5. Compare the onset and severity of catatonic response in the both groups. Plot a graph, time along the x-axis. Note the difference in the onset and severity of catatonic response in both the groups.

Ex.No:12

STUDY OF ANXIOLYTIC OF DRUGS USING MICE/RAT

Aim:

To study the anxiolytic activity of diazepam in mice using elevated zero maze

Principle:

The elevated zero maze test is a behavioural test of anxiety based on the naturalistic tendency of rodents to avoid open and elevated areas. It is similar to the more widely used elevated plus maze, except that the open and closed arms are arranged circularly , thus eliminating the centre area which removes ambiguity in interpretation of time spend in the central square of the traditional design. The maze is an elevated , white or black, annual having outer diameter of 45 cm and inner diameter 30 cm. The runway ring where the mouse can explore 6cm width, which divided into 4 quadrants , 2 opposing "open" quadrants without walls and 2 opposing closed quadrants. The model is widely used for the elevation of anxiolytic agents belonging to chemically different class of drugs.

Requirements:

Animal : Mice (20-25gm)

Drugs: Diazepam (dose 2 mg/kg. i.p)

Equipment: Zero maze with the dimension

Procedure:

1. Weigh and number the animals . divide them into two groups each consisting of minimum 6 mice. One group is used as control and other drugs treatment.
2. Place the animal individually in the open arm facing towards the closed arm. Start the stop watch and note the following parameters for a period of six minutes.
    - Latency to enter the open arm
    - Average time each animals spends in the open arm
    - Total number of entries in the open arm
    - Stretching into open quadrants

3. Clean the maze thoroughly using tissue paper after each trail.
4. Inject diazepam to the test group. After 30 min place the animals individually as described.

Ex.No: 13

STUDY OF LOCAL ANAESTHETIC PROPERTY BY FOOT WITHDRAWAL METHOD

Aim:

To study the local anaesthetic property of procaine HCL using nerve block anaesthesia by frog foot withdrawal method.

Principle :

Local anaesthetics reversibly block impulse conduction along nerve axon and excitable membranes. Their action is used to produce local or regional anaesthesia and for blocking pain sensation . the local anaesthetic property can be easily studied by using

- Nerve block anaesthesia
- Surface anaesthesia
- Infiltration anaesthesia

Requirements:

Animal : Frog

Drugs: Procaine HCL (1%w/v)

Equipment : Frog board surgical instruments

Procedure:

1. Decereb the frog and Place the frog (100-150 gm) 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 hind legs hang free
5. Immense the right hind leg in the beaker containing 0.1N HCL.
6. Note the brisk reflex withdraw of the leg
7. Wash the immerse leg with flowing water or normal saline.

8. Repeat the same with the left hind leg.
9. Wash the immersed left.
10. Place about 10 ml of 1% w/v procaine Hcl in the abdominal pouch. The sciatic nerve flex is exposed to the local anaesthetic actions of the drugs. allow the drug to act for 5 min.