HANDBOOK OF
PRACTICAL PHARMACOLOGY - I

Suman Kamlesh Rattan
Kedar Prasad Meena
Venu Anand Das Vaishnav

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Handbook of Practical Pharmacology - I

Authored by

Suman Kamlesh Rattan
Department of Pharmacy
A Central University,
Guru Ghasidas Vishwavidyalaya
Bilaspur, Chhattisgarh 495009, India

Kedar Prasad Meena
Department of Pharmacy,
A Central University,
Guru Ghasidas Vishwavidyalaya
Bilaspur, Chhattisgarh 495009, India

&

Venu Anand Das Vaishnav
Department of Pharmacy (Neuropharmacology)
A Central University,
Guru Ghasidas Vishwavidyalaya
Bilaspur, Chhattisgarh 495009, India

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Authors: Suman Kamlesh Rattan, Kedar Prasad Meena and Venu Anand Das Vaishnav

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FOREWORD

Pharmacology is the bedrock of effective clinical practice, translating the science of drugs into tangible patient outcomes. In this rapidly advancing field, access to clear, useful, and application-focused information that improve the comprehension of drug actions, interactions, and therapeutic applications is becoming more and more crucial.

The ***"Handbook of Practical Pharmacology - I"*** is a comprehensive and practical resource designed for the modern learners including students, teachers, and medical professionals. This book offers a methodical and practical approach to pharmacological concepts, complete with key drug profiles, well-structured experiments, and crucial insights into pharmacodynamic and pharmacokinetic ideas. Through the integration of theoretical knowledge and practical applications, this handbook enables students to understand basic pharmacological procedures and their practical applications.

This handbook's clarity, accuracy, and dedication to promoting a deeper comprehension of pharmacology beyond rote memorizing are what make it especially important. For the upcoming generation of healthcare workers, it fosters critical thinking, problem-solving, and evidence-based decision-making skills that are essential.

The authors have done a commendable task by producing a resource that will surely help students grasp the intricacies of pharmacology. I have no doubt that this handbook will be a trustworthy ally on the academic path of future pharmacologists and practitioners, providing them the essential knowledge and practical skills, they need to navigate the complexities of pharmacology with confidence and competence.

Preeti K. Suresh
University Institute of Pharmacy
Pt. Ravishankar Shukla University
Raipur, Chhattisgarh, India

PREFACE

With great pleasure, we present the "Handbook of Practical Pharmacology - I," which is devoted to the teachers and students of this nation's pharmacy institutes. This book was created and edited in compliance with the Pharmacy Council of India's (PCI) "Practical Pharmacology-I" syllabus requirement for the second year (4th semester) B. Pharm. degree in pharmacy, as stated in the "Bachelor of Pharmacy (B. Pharm.) course regulations 2014." The book is broken up into fifteen chapters, which are as follows:

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CCSEA Guidelines

Purpose: The Committee for the Control and Supervision of Experiments on Animals (CCSEA) aims to uphold the humane treatment of animals in experimental research by enforcing strict regulatory oversight and supervision. The committee's primary focus is to minimize unnecessary pain, suffering, and distress, thereby fostering ethical and responsible scientific practices involving the use of animals. By acting as a regulatory body, the CCSEA ensures that the welfare of animals is prioritized and safeguarded in research environments.

Mandate: The mandate of the CCSEA is to ensure strict compliance with ethical guidelines and legal requirements pertaining to animal experimentation. This involves monitoring and

evaluating research practices to ensure that they adhere to the established standards of animal welfare. The committee is tasked with reviewing and approving research protocols, conducting inspections, and providing guidance to researchers to ensure that all experiments are conducted responsibly and ethically.

Principles: The CCSEA operates on a set of core principles aimed at promoting respect for animal life. This includes minimizing pain and distress experienced by animals during experiments. Researchers are required to employ the principles of the Refinement, Reduction, and Replacement (the three Rs) to ensure that animal use is justified, the number of animals used is minimized, and procedures are refined to enhance animal welfare.

Scope: The guidelines and oversight of the CCSEA extend to all researchers and facilities engaged in the use of animals for scientific, educational, and medical purposes. This includes universities, research institutions, and private laboratories. The CCSEA functions as the Institutional Animal Ethics Committee (IAEC) at the local level, providing localized oversight and ensuring that all animal use within institutions is in compliance with national and international standards.

Commitment: The CCSEA is committed to fostering a culture of accountability and ethical responsibility in scientific research. This involves continuous education and training for researchers, promoting transparency in research practices, and encouraging the development and adoption of alternatives to animal testing. The committee is dedicated to advancing science in a manner that respects animal welfare and upholds the highest ethical standards.

Sincere attempts have been made to clearly explain the theoretical parts of the pharmaceutical practical components, accompanied by flowcharts and illustrations. Giving pupils comprehensive knowledge of the subject in an easy-to-understand format is the main goal of this book. The challenges that students typically encounter have been taken into consideration. The book’s noteworthy aspects are:

1. It covers every subject listed in the Pharmacy Council of India's "Bachelor of Pharmacy (B. Pharm) course regulations 2014."

2. The language used is modest and eloquent.

We earnestly believe and apprehend that the brilliant budding students of pharmacy (Degree Program) in all Indian universities will definitely find this compilation extremely useful to provide them with sufficient deliberation, understanding, and in-depth knowledge on the subject.

We are grateful to Editorial Manager Publications, Bentham Science Publishers, Sharjah, U.A.E., for all their efforts in publishing this book.

Suggestions and comments are always welcome, and they shall be gratefully acknowledged.

Suman Kamlesh Rattan
Department of Pharmacy
A Central University
Guru Ghasidas Vishwavidyalaya
Bilaspur, Chhattisgarh 495009, India

Kedar Prasad Meena
Department of Pharmacy,
A Central University,
Guru Ghasidas Vishwavidyalaya
Bilaspur, Chhattisgarh 495009, India

&

Venu Anand Das Vaishnav
Department of Pharmacy (Neuropharmacology)
A Central University, Guru Ghasidas Vishwavidyalaya
Bilaspur, Chhattisgarh 495009, India

CHAPTER 1

Introduction to Experimental Pharmacology

OBJECTIVE

To study the general introduction of pharmacology and experimental pharmacology.

Pharmacology is a branch of science that is concerned with the investigation of medications. The word "pharmacology" originates from the Greek words "*Pharmakon*" (a drug or poison) and "*logos*" (discourse). It provides information on the genesis, background, physiochemical properties, and physiological properties, as well as the mechanism of action, distribution, metabolism, excretion, and absorption of drugs. Chemicals known as drugs are employed in the diagnosis, treatment, and prevention of disease in both mammals and humans. The word "drug" originates from the French word "*drogue*" meaning "herb." Within basic medical sciences, the field of experimental pharmacology is relatively recent. Advancements in electrophysiology, biochemistry, molecular biology, and the use of digital recording equipment and software have increased and improved the potential for pharmacology in experimental studies.

The major targets of experimental pharmacology are:

1. To identify a treatment drug that is suitable for individual usage
2. To investigate the toxicity of a drug
3. To investigate the mechanism of action of drugs

There are two primary phases of experimental pharmacology, as it entails finding new therapeutic agents or analyzing how already existing ones work [1].

Preclinical experimental pharmacology is the study of novel chemical structures, their identification and optimization, and their biological activities in animal tissues or organs.

Clinical pharmacology is the study of pharmacological safety, effectiveness, and pharmacokinetics in humans through the testing of pharmaceuticals on patients and volunteers.

Pharmacokinetics is the study of drug distribution, metabolism, excretion, and absorption, which shows the effects of medications on the body [2].

Pharmacodynamics is an exploration of the drugs' mechanisms of action and sites of action, or what the drugs do to the body [3].

Absorption is the intake of a drug that enters the bloodstream or systemic circulation at the point of administration, which shows a systemic action.

Distribution is the traveling of a drug from the systemic circulation to different organs, tissues, muscles, fat, and so forth.

Metabolism is the transformation of a drug into its excretory form.

Elimination is the process of removal of a drug from the body.

Bioavailability is the percentage of an administered dose of an unaltered medication that enters the bloodstream.

Drugs' active components are helpful in the diagnosis, treatment, mitigation, and prevention of any illness or condition in both humans and animals.

Medicine is a material that contains lubricants, binders, sweeteners, and other additives along with the active component and is used to deliver drugs in a stable and acceptable form.

Neuropharmacology is the study of the effects of medication on the functioning of the central and peripheral nervous systems [4].

Pharmacogenetics is the clinical testing of genetic variation that gives rise to differing responses to drugs.

Posology is the study of drug dosage; it depends upon various factors like age, climate, weight, sex, and so on [5].

Pharmacovigilance is described as the study and practice of identifying, evaluating, and comprehending negative impacts.

Side effects are unintended but predictable consequences of a medication or medical procedure.

Adverse effects are secondary effects that are defined as typically undesirable and unsuitable responses.

CONCLUSION

The experiment provided a comprehensive introduction to experimental pharmacology, equipping students with theoretical and practical knowledge of drug evaluation using laboratory models. By exploring key concepts like animal model selection, drug administration, and data interpretation, students can gain insights into the methodologies used to study pharmacological effects. The ethical aspects, such as humane treatment of animals and compliance with CCSEA guidelines, were emphasized to ensure responsible research practices. The introduction to instruments like the Rota Rod and organ bath demonstrated their relevance in assessing the actions of drugs. This foundational experiment underscored the importance of experimental pharmacology in bridging the gap between basic research and clinical application. It will prepare students for advanced studies in pharmacology, emphasizing accuracy, reproducibility, and ethical considerations essential for conducting meaningful preclinical research.

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CHAPTER 2

Instruments Used in Experimental Pharmacology Laboratory

OBJECTIVE

To study the commonly used instruments in experimental pharmacology.

Despite the rapid advancements in electronic equipment and recording systems, research laboratories and institutions still employ them. In experimental pharmacy, certain standard tools are utilized. Even with the leaps in electronic equipment and recording systems, traditional tools continue to hold their ground in research labs and institutions. In experimental pharmacology, several standard tools are essential for the precise and accurate conduct of experiments.

Actophotometer

An actophotometer is a tool used to quantify the locomotor activity of small animals, most commonly rats, in behavior pharmacology (Fig. **1**) [1]. It is an essential technique for pharmacological research, especially when examining how different drugs affect the central nervous system (CNS). When an animal walks through the chamber, the infrared light beams within the chamber are disrupted. The photoelectric sensors translate the incident into an electronic signal and identify each disruption. The signals are routed through a computerized counter, which logs each disruption as an activity unit. The animal's locomotor activity is measured as the total number of interruptions over a given time period.

Rota rod Apparatus

The rotarod test is a behavioral assessment that utilizes a rotating rod to evaluate motor function in rodents (Fig. **2**) [2]. The test gauges important factors, including balance duration (in seconds) and sustained performance. The primary objectives of the test apparatus are to evaluate balance, grip strength, and motor coordination in subjects, particularly in the context of experimental drug trials and post-traumatic brain injury assessments in pharmacological studies.

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Fig. (1). Actophotometer.

Fig. (2). Rota rod apparatus.

Electro-Convulsometer

Electrical stimulation causes seizures, which are subsequently inhibited by systemic anticonvulsant medication (Fig. **3**) [3]. It is possible to study various forms of epilepsy in animal models in labs. Maximum electroshock causes a convulsion in the lab animals. The categories of MES convulsions are tonic flexion, tonic extensor, tonic fission, clonic convulsion, stupor, and recovery/death.

Fig. (3). Electro-Convulsometer.

Eddy's Hotplate

As a source of pain or a stimulant, we use the hotplate. A hotplate is an apparatus with an outside surface and a plate that is heated by an inside heating coil (Fig. **4**) [4]. Mice are the most suitable animal species for this experiment. Mouse species are singled out on this device, which maintains temperature; an analgesic extends the duration in a steady manner using a regulator. Preheating surface with a solid-state and microcontroller-based temperature controller for digital temperature indication allows for precise setting of surface temperature. Animal responses like jumping or licking their paws are recorded at a constant temperature of 550 degrees Celsius. Eddy's hot plate test is used for the evaluation of the analgesic effect of drugs.

CHAPTER 3

Study of Common Laboratory Animals

OBJECTIVE

To study about common laboratory animals.

Mouse (*Mus musculus*)

Surplus albino mice (*Mus musculus*) are frequently employed (Fig. **8**) [1] as they are comparable, affordable, tiny, and manageable.

Fig. (8). Mouse (*Mus musculus*).

Common behavior:

- Timid
- Social
- Territorial
- Nocturnal
- Rarely aggressive when handled properly

Adult weight range for experimental use is 20 to 25 gm; two months is the minimum age for experimentation.

- Acute and sub-acute toxicity are the focus of toxicology studies.
- Insulin bioassay
- Analgesic screening
- Research pertaining to cancer and genetics

Rat (*Rattus norvegicus*)

Wistar strain rats are frequently employed (Fig. **9**) [2]. Commonly employed strains include Sprague-Dawley rats.

Fig. (9). Rat (*Rattus norvegicus*).

- Because of the diminutive size in relation to other animals, a tiny amount of medication is given.
- The drug can be administered orally because of the lack of a vomiting center.
- The tonsils and gall bladder are absent.
- Pancreatectomy is difficult to achieve because of the widespread nature of the pancreas.
- A distinct line exists between the stomach's fundus and pylorus sections.

For experimental use, adult weight range is 200-250 mg; one and a half months of age is appropriate for trials.

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

Guinea pig (*Cavia porcellus*)

The guinea pig is calm and particularly vulnerable to tuberculosis and anaphylaxis. Theyreact strongly to histamine and penicillin (Fig. **10**) [3].

Fig. (10). Guinea pig (*Cavia porcellus*).

Experimental use: The adult weight range for the experiment is 400-600g; the age range is two months

- Evaluation of bronchodilators
- Studies on immunology
- Studies on the oestrus cycle
- Studies on mast cells

CHAPTER 4

Maintenance of Laboratory Animals as per CCSEA Guidelines

OBJECTIVE

To study the maintenance of laboratory animals as per CCSEA guidelines.

The objective of Good Laboratory Practices (GLP) for animal facilities is to ensure the welfare and high standard of care for the animals utilized in research studies.

Goal

These guidelines aim to support the humane treatment of animals utilized in behavioral and biological investigations and testing, primarily by offering guidelines that will improve animal welfare and quality in the effort to expand biological information relevant to both humans and animals [1].

Veterinary Care

- It is the duty of a veterinarian or someone with knowledge or expertise in laboratory animal sciences and medicine to offer adequate veterinary care.
- Someone other than a veterinarian must observe animals on a daily basis; but, a system of open and continuous communication ought to be implemented to ensure that timely and reliable information on issues with animal health, behavior, and well-being is sent to the attending veterinary physician.
- Additionally, veterinarians can also assist the organization in creating suitable policies and procedures for secondary veterinary services, like the application of suitable disease prevention and control techniques (*e.g.*, vaccination and prophylaxis, disease surveillance and observation, seclusion, and monitoring), surgical and post-operative treatment, diagnosis, and management of both injuries and illnesses. They can also do this by analyzing proposals and procedures, animal husbandry, and the well-being of animals, as well as keeping an eye on the containment of occupational health risks, developing initiatives to control zoonosis, and overseeing animal care and cleanliness. Established conditions dictate whether full-time, part-time, or consultative veterinarian care is required [2].

Acquisition of Animals

All animals (including sheep, goats, cattle, buffalo, pigs, and horses, among others) must be legally obtained in accordance with the CCSEA regulations. Dogs and small animals can be obtained from licensed breeders. Large animals can be obtained from farmers, or under the direction of the department of wildlife, similar to how macaques are treated. Cats are useful for breeding purposes. It is possible to transport rats from overseas following the acquisition of the required Director General of Foreign Trade (DGFT) license for import. Animal quality should be evaluated before purchasing by conducting a health surveillance program for purchased animals. It is also important to consider the modes of transportation. Animals should be quarantined and stabilized in accordance with protocols suitable for the species and situation, and every shipment of animals should be examined to ensure that procurement requirements are met [3].

Quarantine, Stabilization, and Separation

- When an animal is placed in quarantine, it is kept apart from other animals in the facility until its health and maybe its microbiological status are established. An efficient quarantine reduces the possibility of germs entering an established colony. Small laboratory animals are quarantined for one week to one month, while larger animals (cats, dogs, monkeys, *etc*.) are quarantined for up to six weeks. However, the duration depends on the kind of infection or probable infection found in animals.
- To assist in reducing human exposure to zoonotic illnesses, non-human primates should be placed under effective quarantine. Depending on how the TB test turns out, the duration can range from two to three months. Worldwide, macaques that test positive for tuberculosis at least twice or those who exhibit symptoms of illness or weight loss are euthanized to avoid transfer of tuberculosis to employees and other macaques.
- Newly arrived animals should be allowed a period for physiological, psychological, and nutritional stability before being used, regardless of the length of the quarantine. The period and type of animal transportation used, the species involved, and the stabilization period will all affect how long it takes, as well as the animals' intended purpose.
- To prevent the spread of diseases between species, reduce anxiety, and avoid any physiological and behavioral changes brought on by interspecies conflict, it is advised that animals be physically separated by species.
- Different species are typically housed in distinct rooms, cages with filtered air or separate ventilation, and isolators. It is permissible in certain cases to keep different species in the same room, such as when they are behaviorally compatible and share a comparable pathogenic status. Other workers should be

prohibited from entering the facility unless absolutely necessary, and once they have handled these sick animals, they should not handle any other animals there. A separate set of professionals should be designated to care for these diseased animals.

Monitoring, Diagnosis, Therapy, and Disease Control

- Animal house workers should keep an eye out for any signs of disease, injury, or unusual behavior in all of the animals. This should ideally happen every day, although there are times when more frequent observations are necessary, such as after surgery or in cases where the animal is unwell or physically deficient. Appropriate techniques for illness surveillance and diagnosis must be implemented.
- In order to guarantee the proper and timely provision of veterinary medical treatment, post-mortem examination should be done whenever an animal exhibits signs of disease, suffering, or other abnormalities in their general health as soon as possible. Animals that exhibit symptoms of a communicable illness ought to be kept apart from the colony's healthy animals. When non-human primates are exposed to an infectious pathogen, such as *Mycobacterium tuberculosis*, the group should be segregated and maintained together for diagnosis and treatment to control the spread of the disease. Animals with infectious illnesses, such as tuberculosis, *etc*., must be put to death immediately to stop them from spreading to other animals and even to animal handlers.
- For newly arrived animals, isolation, quarantine, and stabilization programs are required to give time for a health status evaluation, help them recuperate from the stress of transportation, and provide them time to become acclimated to their new environment. The scope of these initiatives is determined by several elements, such as the animals' species, origin, and intended purpose. For certain animals, such as rodents that come from trustworthy sources and whose health status is known, a quick examination upon arrival might be sufficient. For animals, such as agricultural animals, nonhuman primates, wild creatures, canines, rodents, and rabbits, that are free of particular pathogens, it is necessary to follow isolation and quarantine protocols.
- Vaccinations, treatments for ecto- and endoparasites, and other disease management methods are examples of preventive medicine programs that should be started in accordance with current veterinary medical standards suitable for the specific species and source.
- Animals that are transgenic or mutant may be more vulnerable to illness and may need more care to maintain their health. Standard operating procedures, containment/isolation devices, and facility design elements are a few examples of systems used to stop the transmission of illness. To stop the spread of animal diseases, personnel involved in research and animal care must receive proper

CHAPTER 5

Commonly Used Laboratory Techniques

OBJECTIVE

To study common laboratory techniques used in animal experiments.

ANESTHESIA IN EXPERIMENTAL ANIMALS

Anesthesia: The loss of feeling, usually brought on by damage to a nerve or receptor, but it can also result from medication use or other medical procedures. **Analgesia:** Pain relief

Tranquilization: A behavioral shift in which the animal exhibits relaxed, environment-indifferent, and frequently pain-indifferent behavior.

Sedation: It causes the animal to be alert and peaceful while having a mild case of CNS depression. Loss of sensation in a specific location is known as local anesthesia.

Insensibility: Anesthesia in a broader but still constrained region.

Basal Anesthesia: A mild form of general anesthesia caused by a pre-anesthetic drug that gets the animal ready for the administration of more drugs or a deeper anesthesia.

General Anesthesia: Total unconsciousness with general anesthesia.

Surface Anesthesia: A state of consciousness combined with a degree of muscle relaxation that permits painless operation. For instance, proparacaine and tetracaine.

Injectable Local Anaesthetics: For gentle and peaceful animals (cattle, sheep), injectable local anesthetics such as proparacaine, lidocaine, mepivacaine, and etiodocaine are used. For the majority of laboratory animals, general anesthesia is the preferred approach. Animals should only be used in experiments for biomedical research if they are cognizant. It is not feasible to conduct the study while the animal is sedated. Conditions for anesthesia

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should always be selected with the intention of minimizing pain, discomfort, and tension as potential harmful factors on the repeatability of the data and the pharmaceutical outcomes.

GENERAL ANESTHESIA

Preparation: The animal should be made to fast for 12 hrs (Water-fasting ad libitum).

Premedication: To facilitate the administration of the anesthetic and minimize its negative effects. For example, atropine is administered intramuscularly (IM) before general anesthesia to prevent cardiac complications and to reduce salivation (Table **1**) [1].

Table 1. Various medications.

Species	Premedication	Sedation	Short Anaesthesia	Medium Anesthesia	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 Phenobarbitone (50 i.p.)	Xylazine + Ketamine (16+100 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 Phenobarbitone (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 Phenobarbitone (35 i.p.)	Xylazine + Ketamine (10+200 i.m.)
Guinea Pig	Atropine (0.1-0.2 s.c.)	Diazepam (2.5-5 i.p.)	Inhalation (Isoflurane)	Xylazine + Ketamine (2+80 i.m.)	Xylazine + Ketamine (4+100 i.m.)
Rabbit	Atropine (0.1-0.2 s.c.)	Diazepam (1-5 i.p.)	Inhalation (Isoflurane)	Xylazine + Ketamine (5+25-80 i.m.)	Xylazine + Ketamine (5+100 i.m.)

Xylazine - Administered IM to make the animal calm, dilates the blood vessels.

COURSE OF ANAESTHESIA

Four Stages of Anesthesia

- ***Stage of analgesia (from the first effect to unconsciousness):*** Increased heart and breathing rates, together with typical pupil dilatation.

- ***Stage of excitation (from the beginning of unconsciousness to the start of regular respiration):*** Irregular breathing, dilated pupils, heightened motor function, reflexes, nystagmus,
- ***Stage of tolerance (from the beginning of regular respiration to the termination of spontaneous respiration):*** Small pupils, relaxed skeletal muscles, evident corneal reflex, and absence of eyelid reaction, flat breathing, and effective analgesia.
- ***Stage of asphyxia (after termination of the spontaneous diaphragmatic respiration):*** Absence of breathing and reflexes poses a risk of mortality, so using antidotes right away is essential to preventing it.

GENERAL ANESTHESIA INDUCTION ROUTES

There are two main approaches to general anesthesia induction.

Injection and Inhalation

- ***Injection:*** The narcotic chemical dissolves in a liquid when administered *via* this method. IV, IM, SC, or IP may be the administration route. Below is a list of the compounds that are most often used:

- Barbiturates- Phenobarbitone, thiopental-sodium
- Chloral hydrate
- Ketamine
- Hypotonic agents- Mithomidate
- Xylazine
- Urethane

Inhalation: For small laboratory animals like rats, this type of anesthesia is largely ineffective. Larger laboratory animals, including dogs, cats, monkeys, sheep, and goats, are more likely to experience it. The benefits of this anesthetia induction route are the options to precisely regulate the level of anesthetic and the speed at which issues are handled.

PROCEDURE

Rat anesthesia: Xylazine–Ketamine

- Combine 1.25 mL of Xylazine (100 mg/mL) and 8.75 mL of Ketamine (100 mg/mL) in a sterile, 10-milliliter vial with a rubber stopper. Shake well prior to use.
- Keep in a cool, dark area away from light.

CHAPTER 6

Study of Different Routes of Drug Administration in Murines

OBJECTIVE

To study the different routes of drug administration in murine.

PROCEDURE

The following procedures given below are the different routes of drug administration in mice and rats:

Intraperitoneal Injection

- First, the needle's entrance point was found. An imaginary line was drawn above the knees across the abdomen (Fig. **20**) [1].
- A needle was put along the midline, which is on the right side of the animal.
- The point of entry for a female rat or mouse is cranial to the last nostril and slightly medial to it.
- The cecum, a bigger organ on the left side of the abdomen that is packed with fluid, is avoided when the needle is inserted on the right side of the mouse.
- Because the injection might harm the muscle in the back of the leg, it was best to avoid inserting the needle too deeply or laterally from the insertion point. The muscle must be tightly gripped to prevent movement throughout the treatment.
- The mouse needs to be securely confined in order to prevent movement during the IP injection operation.
- The mouse was tilted and constrained such that its abdomen was visible and its head was looking downward. After cleaning the injection site, the needle was placed into the abdomen at a 30-degree angle.
- The needle shaft should be inserted approximately half a centimeter below the surface. To ensure that the needle had not pierced a blood vessel, the intestines, or the bladder, it was aspirated.
- A greenish brown aspirate shows that the needle entered the intestines.
- The process was repeated using a fresh syringe and needle if any fluid was aspirated. The contaminated solution had to be thrown away.

- The injection was administered if no fluid was aspirated. After taking out the needle, the mouse was put back in its cage.
- IP needle size 25–27 G is advised.

Fig. (20). Intraperitoneal injections.

Precautions for Intraperitoneal Administration in Rats and Mice

Intraperitoneal (IP) administration in rats and mice is a common technique in experimental research, but it requires careful attention to ensure the safety and well-being of the animals. Some key precautions to keep in mind are as follows:

- **Training and Competency:** Only personnel trained and deemed competent in IP injection techniques should perform the procedure.
- **Volume and Concentration:** The volume of the substance injected should be the lowest possible and not exceed the recommended guidelines. For mice, the maximum volume is typically less than 0.5 ml/kg, and for rats, it is less than 2 ml/kg.
- **Needle Size:** Use the appropriate needle size for the species and substance. For mice, a 25-27 gauge needle is recommended, and for rats, a 23-25 gauge needle is suitable.
- **Aseptic Technique:** Maintain aseptic conditions to prevent infection. Use sterile substances, and disinfect the injection site with 70% isopropyl alcohol.
- **Substance Temperature:** Warm the substance to room or body temperature before injection to avoid discomfort and shock.
- **Restraint:** Properly restrain the animal to minimize stress and ensure accurate

injection. For mice, tilt the head slightly downward; for rats, use a gentle but firm hold.
- **Monitoring:** Observe the animals closely after injection for any signs of adverse reactions or complications.

Additional Considerations

- **Frequency of Injections:** Limit the frequency of IP injections to reduce stress and potential complications.
- **Justification:** Provide justification for using IP administration over other routes, as it can be less reliable and more invasive.
- **Ethical Approval:** Ensure all procedures are approved by the relevant animal care and ethics committees.

Subcutaneous Injection

- The skin was raised to create a tent after the mouse was restrained as usual. After cleaning the injection site, the needle was inserted into the subcutaneous tissue (Fig. **21**) [2].
- Before administering the injection, the needle was aspirated; if no aspirate appeared, confirming the correct position, the injection was administered.
- The injection location was chosen to be the area of loose skin around the neck and shoulders.
- Subcutaneous injections were primarily used to inject anesthetics and provide fluids for hydration.
- Average volumes of 1 milliliter or less of the injected area.
- A tent-like structure of folded skin covered the back.
- To prevent puncturing underlying structures, the needle was put at the base of the tent while being held parallel to the animal's body.
- Awoken mice were injected by placing them on the wire lid, allowing them to hang with their front paws during the process. There were scratches on the back skin, and a tent was constructed. A hand was used for scuffing, and a tent was made for both presentation and restraint of the injection site.
- To make sure the needle had not gotten into a blood vessel, it was aspirated.
- The injection rate was modest for the entire volume.
- After removing the needle, the skin was compressed to close the needle's exit hole and stop medication leakage.
- The animal was examined for signs of bleeding.
- A fluid deposit was made in the subcutaneous area, allowing for the visual and tactile perception of the BELB bubble.
- SC needle size 23–25 G is advised.

CHAPTER 7

Effects of Hepatic Microsomal Enzyme Inducers on Phenobarbitone-Induced Sleep Duration in Mice

OBJECTIVE

Study the effect of hepatic microsomal enzyme inducers on the phenobarbitone-induced sleeping time in mice.

REQUIREMENTS

Animals: Mice.

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

Microsomal Enzyme Inducers and Their Types

Microsomal enzyme inducers are substances that enhance the activity and expression of microsomal enzymes, particularly those of the **cytochrome P450 (CYP)** enzyme family, in the liver and other tissues. These enzymes are crucial for the metabolism of drugs, endogenous compounds (*e.g.*, steroids), and xenobiotics [1].

Types of Microsomal Enzyme Inducers

Microsomal enzyme inducers can be broadly categorized based on their chemical nature or the specific enzymes they induce [2].

Pharmacological Inducers

These are drugs that enhance the activity of microsomal enzymes:

- **Rifampin:** A potent inducer of CYP3A4 and other enzymes.
- **Carbamazepine:** Induces CYP3A4, CYP1A2, and CYP2C9.
- **Phenytoin:** Induces CYP3A4, CYP2C9, and CYP2C19.
- **Phenobarbital:** Induces a wide range of CYP enzymes.
- **Dexamethasone:** Induces CYP3A4 and CYP2C enzymes.

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Dietary and Herbal Inducers

- **St. John's Wort:** Induces CYP3A4 and P-glycoprotein, affecting drug bioavailability.
- **Cruciferous Vegetables (*e.g.*, broccoli, cabbage):** Contain indoles that induce CYP1A enzymes.
- **Grapefruit:**Induces paradoxical effect depending on compounds.

Environmental Inducers

- **Polycyclic Aromatic Hydrocarbons (PAHs):** Found in tobacco smoke and charred meat; they induce CYP1A enzymes.
- **Pesticides and Herbicides:** Induce enzymes involved in xenobiotic metabolism.

Endogenous Compounds

- Certain **hormones** or physiological states can lead to enzyme induction. For example, **glucocorticoids**, which induce CYP3A enzymes.

Synthetic and Industrial Chemical

Alcohol: Chronic alcohol consumption induces CYP2E1, which metabolizes ethanol and other toxins.

CLINICAL IMPLICATIONS OF ENZYME INDUCTION

1. **Altered Drug Metabolism:**
   - Enhanced metabolism may reduce drug efficacy (*e.g.*, oral contraceptives).
   - Increased formation of toxic metabolites in some cases (*e.g.*, acetaminophen and CYP2E1).
2. **Drug Interactions:**
   - Concurrent use of enzyme inducers with other medications may necessitate dose adjustments.
3. **Disease Impact:**
   - Conditions like porphyria can worsen due to increased heme synthesis.
4. **Environmental Exposures:**
   - Prolonged exposure to environmental inducers (*e.g.*, smoking) affects the metabolism of many drugs [3].

PRINCIPLE

Substances that activate the hepatic microsomal oxidative enzyme system improve how well other substances are metabolized. Consequently, the duration of the effect of the second medicine will be diminished in the presence of an enzyme inducer. This has important therapeutic implications because when

multiple medications are given concurrently, one medication may induce the activity of microsomal enzymes and one can alter the activity of another. Typical medications that trigger the hepatic microsomal enzyme system include Meprobamate with phenobarbitone. Any medication administered in combination with any of these medications may have an impact on how the second drug is disposed of, and thus, the intended pharmaceutical outcomes.

PROCEDURE

- Weigh and number the animals. Split them up into two groups of no fewer than six mice each.
- Give the first group one injection of phenobarbitone every day for five days. Give the other group the same distilled water for five days.
- On the fifth day, inject Phenobarbital into both groups one hour after the previous dosage of phenobarbitone.
- Observe how long the phenobarbitone-induced sleep lasted in each group and when it started.

INFERENCE: In contrast to animals that received distilled water treatment, animals pretreated with phenobarbitone slept for shorter periods of time.

REPORT: A study was conducted on the impact of hepatic microsomal inducers on the sleeping period of mice treated with phenobarbitone.

CONCLUSION

The results of the experiment demonstrated that hepatic microsomal enzyme inducers considerably cut down on the amount of time that mice spent sleeping as a result of phenobarbitone. This finding lends credence to the hypothesis that enzyme induction speeds up the metabolism of the drug. The hepatic enzyme activity was increased by pre-treatment with medicines such as phenobarbital and rifampicin, which resulted in a quicker clearance of phenobarbitone from the system and a shorter duration of drowsiness. In addition to shedding light on the significance of hepatic microsomal enzymes in the process of drug metabolism, these findings offer valuable insights that can be utilized to better comprehend the interactions between drugs, particularly in the context of enzyme enhancement. The study highlights the possibility of altered pharmacological efficacy due to changes in drug metabolism, which has substantial implications for clinical pharmacology. These implications are especially relevant when it comes to the management of patients who are undergoing long-term therapy with enzyme inducers and barbiturates.

CHAPTER 8

Drugs Acting on Ciliary Motility of Frog Esophagus

OBJECTIVE

To study the effects of drugs on the ciliary motility of the frog esophagus using the ExPharm T2 version.

PRINCIPLE

Cilia are found in the frog esophagus. The function of acetylcholine in the mucous membrane is necessary for ciliary motion. ACh stimulates cilia to contract, which increases movement. Anti-cholinergic medications immobilize cilia and have comparable effects to cholinergic medicines, which lessen their motion. To illustrate, this experiment uses a handful of these medications to obtain their result [1].

REQUIREMENTS

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

Drugs and Solutions

a. Frog ringer (NaCl 8g/L, KCl 0.2g/L, $CaCl_2$ 0.1g/L , $MgSO_4$ 0.1g/L)
b. Physostigmine 10%
c. Atropine 0.1%
d. Acetylcholine 10%

SET UP: A frog's lower jaw is extracted together with its pith. An opening is made in the esophagus from the buccal cavity to the stomach. It iseverted and pinned onto a wooden board. Blood is eliminated from frogs by using a cotton swab soaked in Ringer's solution. The surface is dampened with Ringer's solution, and a poppy seed is positioned at the head's end. In the esophagus, motions and the amount of time needed to traverse a certain distance are noted (Fig. **26**) [2].

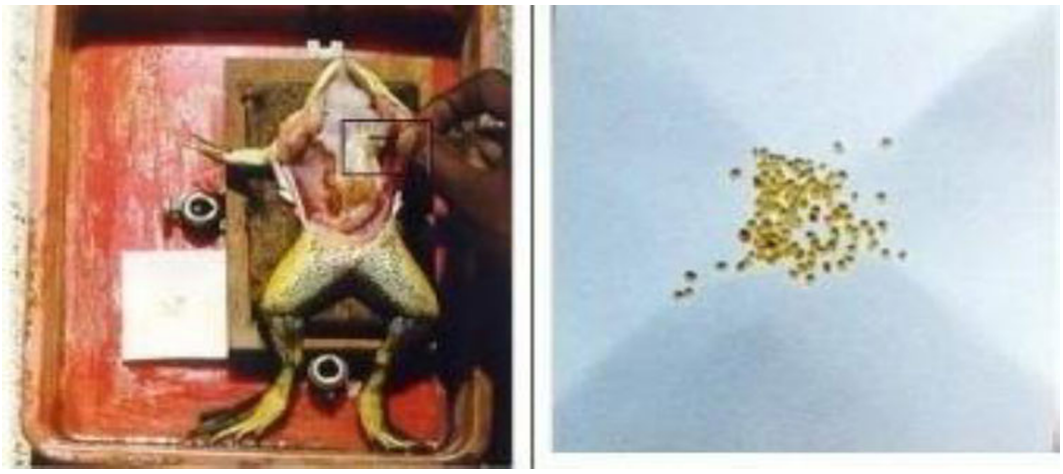

Fig. (26). A Poppy seed being placed in the esophagus.

PROCEDURE

1. Calculate the seed's migration distance. Pins positioned at the caudal (distal) end and the cephalic end, respectively, will serve as the beginning and ending points.
2. Apply Ringer's solutionto the esophageal surface. At the cephalic end of the esophagus, plant a poppy seed. Because of ciliary motility, the seed begins to move. Start the stopwatch when the seed passes the cephalic end pins, which serve as the beginning point. When the seed reaches the distal pins, stop the watch.
3. Record how long it takes for the seed to cover the distance. To receive three readings, repeat step two again.
4. Take three readings after administering ACh.
5. Carry out steps two and three again.
6. Take three readings after administering physostigmine.
7. Carry out steps two and three again.
8. Take three readings after administering atropine.
9. After step 6, instill ACh and see its impact (without utilizing frog-Ringer). Compare it to the result achieved using ACh alone (Step 4).
10. Write conclusions after tabulating your readings.

NOTE

1. Determine the average reading for each drug after testing it three times, including that for Ringer's solution.
2. Readings for Ringer's solution are used as a control and contrasted with test (drug) readings.
3. Prior to testing any drug, obtain readings using Ringer's solution and take

separate control readings for each substance.
4. Use new preparations (frog) for each drug. Drugs should be applied to the same preparation (frog) consecutively without using Ringer's solution in between in order to observe interactions.

To ensure accurate and reliable results when testing the effects of different drugs using frog preparations, a systematic approach is essential. Firstly, for each drug, the average reading should be determined after conducting three tests, including that for the Ringer's solution. The readings for Ringer's solution serve as a control and compared against the test readings for each drug. Before testing any drug, control readings using the Ringer's solution must be obtained for each substance individually. It is crucial to use a new frog preparation for each drug. Drugs should be applied consecutively to the same preparation without interspersing with Ringer's solution in between to observe any potential interactions between the drugs. This method allows for accurate comparisons and the observation of drug interactions, ensuring the reliability and validity of the experimental results.

OBSERVATION

REPORT

A study was conducted to study the effects of drugs on the ciliary motility of frog esophagus (Table **3**) using Expharm T2 version.

Table 3. Effect of drugs on the ciliary motility of frog esophagus [3].

S.No.	Drugs	Reading 1	Reading 2	Reading 3	Mean
1.	Ringer	-	-	-	-
2.	Acetylcholine	-	-	-	-
3.	Physostigmine	-	-	-	-
4.	Atropine	-	-	-	-

CONCLUSION

During the course of the experiment, it was successfully established that different medicines have different effects on the ciliary motility of the esophagus of the animal. It is clear that acetylcholine has a function in promoting ciliary activity through cholinergic pathways because it was found to enhance the frequency of ciliary beats. The effects of adrenaline were contradictory, which suggests that it has a complex relationship with adrenergic receptors. On the other hand, local anesthetics such as procaine decrease ciliary motility, most likely because of the

CHAPTER 9

Impact of Several Drugs on the Rabbit Eye

OBJECTIVE

To study the effect of drugs on the rabbit eye.

PRINCIPLE

Many medications are applied topically as ointments or drops to target specific areas of the eye. The majority of these medications fall into the autonomic, local anesthetic, or antimicrobial pharmacological classes. Nerves that supply the eye are both sympathetic and parasympathetic. The dilator papillae of the iris and the superior palpebral muscle are supplied sympathetically. The parasympathetic nervous system controls the sphincter pupillae of the iris. Furthermore, as the parasympathetic nerve contracts, the ciliary muscle flexes inward and forward, receiving its supply from the ciliary body. The eye is adapted for close vision because of the forward protrusion of the lens. On the other hand, the paralysis of the accommodation process is brought about by the relaxation of the ciliary muscle. Drugs given topically can alter the cornea's sensitivity, light reflex, intraocular pressure, conjunctival congestion, and papillary size. Nonetheless, students may readily research how drugs affect corneal reflex, light reflex, and papillary size. A clear plastic scale can be used to measure the eye’s pupil size by positioning it in front of the eye as close as feasible. A torch's light pointed at the pupil will cause a light reflex. A sterile thin cotton swab is used to gently contact the cornea from the side so that the patient does not see it. This causes a corneal reflex, which causes the crystals to blink.

A mydriatic is a type of medication that causes the pupil of the eye to dilate (widen). Mydriatics are often used during eye examinations to allow the ophthalmologist or optometrist to get a better view of the inside of the eye, including the retina and optic nerve. These medications can also be used in the treatment of certain eye conditions, such as uveitis, where dilating the pupil can help alleviate pain and prevent the formation of adhesions within the eye.

Common mydriatic agents include:

- **Atropine**
- **Tropicamide**
- **Phenylephrine**

Miosis:

Miosis refers to the constriction of the pupil of the eye. It occurs when the muscles in the iris contract, making the pupil smaller. Miosis can be a normal physiological response to bright light, as the eye adjusts to protect the retina from excessive light exposure. It can also be induced by certain medications, such as miotic drugs, or occur due to various medical conditions.

Common causes of miosis include:

- **Exposure to bright light**
- **Certain medications** (*e.g.*, pilocarpine)
- **Opioid use**
- **Neurological conditions**
- **Eye injuries**

REQUIREMENTS

Animal: Rabbit (2-5kg); Drugs: Atropine (1%w/v) and Physostigmine (1%w/v)

EQUIPMENT

rabbit holder, pen torch

PROCEDURE

1. Keeping the head outside, place the rabbit in the rabbit holder.
2. Examine the size of the pupils in each eye.
3. Hold the torch in front of the rabbit’s eyes and move the light beam to see the effects of light reflex.
4. Examine the corneal reflex by using a cotton swab to touch the cornea.
5. As a control, inject a few drops of atropine solution into the rabbit's right eye's conjunctiva over the course of eight to ten minutes. Fill the left eye with regular saline.
6. After the drug has been initialized for ten minutes, measure the pupil size, light reflex, and corneal reflex, then tabulate the results.
7. Conduct the same trials using ephedrine and physostigmine.

Table 4. Effect of Different Drugs on Pupillary Size, Light Reflex, and Corneal Reflex.

Drug	Pupillary Size	Light reflex	Corneal reflex
Saline	Normal	Present	Present
Physostigmine	Constriction	Present	Present
Ephedrine	Dilation	Present	Present
Atropine	Dilation	Absent	Present

INFERENCE

Saline: Instilling saline has no effect on pupil size. Light reflexes are present.

Physostigmine: Reduces iris diameter and pupil size, resulting in miosis. Reflexes to light and touch are present.

Ephedrine: Propecia enlarges the pupil and dilates the iris, resulting in mydriasis. The light reflex is still lacking, but the touch reflex and light reflex are present.

Atropine: Increases the pupil size and the diameter of the iris, thereby causing mydriasis.

While there is a touch reflex, there is no light reflex. Table **4** is followed by the reference [1].

CONCLUSION

The experiment successfully illustrated the impacts of different medications on the ocular functions of rabbits, shedding light on the roles of atropine, pilocarpine, and tetracaine in ocular pharmacology. Atropine, a mydriatic, causes pupil dilation, while pilocarpine, a miotic, induces pupil constriction. Tetracaine, an anesthetic, effectively blocks reflex reactions. These findings are significant because they underscore the utility of rabbits in ocular drug testing, providing valuable insights into the pharmacological effects of topical medicines used in ophthalmology [2].

The study highlights several important aspects:

1. **Atropine**: This drug is responsible for the dilation of pupils. In ophthalmology, this property is particularly useful for eye examinations and surgeries, allowing better visualization of the eye's internal structures [3].
2. **Pilocarpine**: Known for its ability to constrict the pupils, pilocarpine is essential in treating conditions such as glaucoma. Reducing the pupil size helps

CHAPTER 10

Impact of Skeletal Muscle Relaxants Assessed *via* Rotarod Apparatus

OBJECTIVE

To study the effect of skeletal muscle relaxants using the Rotarod apparatus.

PRINCIPLE

Muscle relaxing ability is one of the key pharmacological actions of anti-anxiety medications in the benzodiazepine class of pharmaceuticals. These substances have a calming or sedative effect, in addition to relaxing skeletal muscles, which helps lower tension and anxiety. One sign of muscle relaxation is a loss of grip. Easily tested on animals, this effect can be measured with an inclined plane or revolving rods. An indicator of muscle relaxation is the difference in the fall-off time of the rotating rod between the animal in the diazepam-treated group and the animal in the control group. It is necessary to adjust the inclined plane's slope angle or the rod's spinning speed so that an average mouse can stay on the plane or on the rod for a significant period of time.

Skeletal Muscle Relaxant

A skeletal muscle relaxant is a type of medication that affects skeletal muscle function and decreases muscle tone. These drugs are used to alleviate symptoms such as muscle spasms, pain, and hyperreflexia [1]. There are two main types of muscle relaxants:

Neuromuscular blockers: These act by interfering with transmission at the neuromuscular end plate and are often used during surgical procedures to cause temporary paralysis.

1. **Spasmolytics (centrally acting muscle relaxants)**: These work within the central nervous system to alleviate musculoskeletal pain and spasms.

REQUIREMENT

Animal: Mice weighing 20–25 grams

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Drugs: Administer 1 milliliter per 100 grams of the mouse's body weight using a diazepam dose.

Equipment: Rota rod apparatus

PROCEDURE

1. Once the animals are weighed, number them.
2. Activate the device and choose a suitable speed (20–25 rpm).
3. Arrange the animals on the revolving rod one by one. More than one mouse may be placed. Record the moment at which the mouse drops from the revolving rod. A typical mouse usually falls off in three to five minutes.
4. Give all animals diazepam injections. Repeat step 3 trials after 30 minutes. Take note of the cutoff time.
5. Examine the animals' fall-off times both before and after receiving diazepam.

OBSERVATION

Dose of Diazepam: 4 mg/kg

The dose of **4 mg/kg of Diazepam** is used in murine studies to evaluate its **skeletal muscle relaxant properties** [2], because it helps achieve a balance between efficacy and safety. Diazepam, a benzodiazepine, exerts its muscle relaxant effects by enhancing the action of GABA (gamma-aminobutyric acid), which is an inhibitory neurotransmitter in the central nervous system1. This action results in decreased muscle tone and relaxation.

Table 5. Effect of Drug Treatment on Fall Time in Mice Following Dose Administration Based on Body Weight.

S. No.	Body weight (g)	Dose to be administered (mg)	Fall time (seconds)		% decrease in fall time
			Basel (A)	Treatment (B)	
1.	25	0.1	60	45	25.00%
2.	30	0.12	58	42	31.03%
3.	32	0.128	62	43	30.65%
4.	35	0.14	63	42	34.38%
5.	33	0.132	64	44	30.16%

% decrease= A-BA×100. This formula isusedto calculate the percentage decrease in fall time. Table **5** is followed by the reference [3].

INFERENCE

As the locomotor activity score decreased in rats given chlorpromazine, it was discovered that the drug had CNS depressive properties.

CONCLUSION

The experiment successfully demonstrated that skeletal muscle relaxants, such as diazepam and baclofen, dramatically impacted the motor coordination and balance of mice. This was demonstrated by a reduction in the amount of time that the rodents spent on the

revolving rod. It was discovered that the Rotarod apparatus is both dependable and sensitive for assessing the effects of skeletal muscle relaxants, which in turn provides significant information regarding the pharmacodynamics of these substances. The purpose of this test is to investigate the processes that are responsible for the activity of possible muscle relaxants and to screen new medicines. The findings have significant repercussions for the creation of therapeutic drugs for illnesses that need muscular relaxation, such as spasticity or muscle spasms, while also taking into consideration the potential adverse effects that are associated with motor coordination.

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CHAPTER 11

Effect of Drugs on the Locomotor Activity of Mice using Actophotometer

OBJECTIVE

To study the effect of drugs on the locomotor activity of mice using an actophotometer.

PRINCIPLE

The majority of medications that affect the central nervous system have an impact on how humans and animals move. Alcohol and other CNS depressants, like barbiturates, lower motor activity, whereas stimulants, like amphetamines and caffeine, boost it. Stated differently, locomotor activity can be indexed as ameasure of mental activity during waking hours. An actophotometer is a simple tool to use to monitor locomotor activity. It uses photoelectric cells that are wired into a circuit with a counter to function. When the light from the animal stops the light from landing on the photocell, a count is made. Actophotometers may have an arena in which the animal moves that can be square or circular. Rats and mice can both be employed in tests [1].

REQUIREMENT

Animal: Mice weighing 20-25 grams

Drugs: Chlorpromazine hydrochloride

Equipment: Actophotometer

PROCEDURE

1. Weigh each animal and assign a number.
2. After turning on the apparatus, put each mouse in the activity cage for ten minutes at a time. Each animal's basal activity score should be noted.
3. After 30 minutes of chlorpromazine injection, retest each mouse for activity scores for ten minutes. Observe how the activity changes before and after the administration of chlorpromazine.
4. Determine the motor activity drop as a percentage. % decrease= A-BA×100

Table 6. The effect of diazepam on locomotor activity in mice: evaluation of dose-dependent cns depression.

S. No.	Body Weight	Dose to be Administered	Basel (A)	Treatment (B)	Difference (A-B)	% Decrease in Locomotor Activity
1.	25	0.075	120	70	50	41.67%
2.	30	0.09	13	65	65	50.00%
3.	32	0.096	125	60	65	52.00%
4.	35	0.105	135	55	80	59.26%
5.	34	0.103	140	58	82	58.57%
6.	33	0.099	128	62	66	51.56%

Dose of chlorpromazine: 3mg/kg. Table **6** is followed by reference [2].

INFERENCE

As the locomotor activity score decreased in rats given chlorpromazine, it was discovered that the drug had CNS depressive properties [3].

CONCLUSION

The research effectively revealed that the central nervous system stimulants like caffeine and amphetamines enhanced locomotor activity in mice, whilst the central nervous system depressant diazepam decreased movement. This finding aligns with the pharmacological effects known to be associated with these substances. The actophotometer was a significant instrument for preclinical screening of medications that could potentially affect motor functions, as it provided a dependable and effective method for monitoring changes in locomotor activity [4]. Not only do these findings confirm the use of this method in evaluating the behavioral effects of medications, but they also highlight the significance of this method in an effort to comprehend the pharmacodynamics of substances that are active in the central nervous system. The findings have wider-ranging implications for the development of drugs, particularly for compounds that are intended to treat neurological or psychiatric diseases, which are conditions in which the modulation of motor activity is highly important.

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CHAPTER 12

Anticonvulsant Effect of Drugs by MES & PTZ Method

OBJECTIVE

To study the anticonvulsant effect of drugs by the MES & PTZ method.

Epilepsy

Epilepsy is a chronic neurological disorder characterized by recurrent seizures, which are sudden and abnormal bursts of electrical activity in the brain. These seizures can vary in type, intensity, and frequency and may result in alterations in behavior, sensation, or consciousness.

Pathophysiology of Epilepsy

Seizures occur due to an imbalance between excitatory and inhibitory neurotransmission in the brain:

1. **Hyperexcitability:** Excessive firing of excitatory neurons due to enhanced activity of glutamate or reduced inhibition by GABA.
2. **Hypersynchrony:** Groups of neurons fire in a highly synchronized manner, leading to abnormal electrical discharges.
3. **Structural or Metabolic Alterations:** Changes in ion channels, neurotransmitter levels, or neuronal circuitry predisposed to seizures.

Types of Epileptic Seizures

The International League Against Epilepsy (ILAE) classifies seizures into two major types:

1. Focal (Partial) Seizures
   - Originate in a specific area of one hemisphere of the brain.
   - **Simple Focal Seizures:** No loss of consciousness; symptoms depend on the affected brain area (*e.g.*, motor, sensory).

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- **Complex Focal Seizures:** Impaired awareness may be accompanied by automatisms (*e.g.*, lip-smacking).

2. Generalized Seizures

- Involve both hemispheres from the onset.
- **Tonic-Clonic (Grand Mal) Seizures:** Characterized by stiffening (tonic phase) followed by jerking movements (clonic phase).
- **Absence (Petit Mal) Seizures:** Brief episodes of staring or loss of awareness.
- **Myoclonic Seizures:** Sudden, brief muscle jerks.
- **Atonic Seizures:** Sudden loss of muscle tone, causing falls.
- **Tonic Seizures:** Sustained muscle contractions.
- **Clonic Seizures:** Rhythmic jerking movements.

Diagnosis of Epilepsy

1. Clinical History
   - Detailed account of seizure events, including triggers, duration, and associated symptoms.
   - Family and medical history.
2. Electroencephalogram (EEG)
   - Records electrical activity in the brain to detect abnormal patterns associated with epilepsy.
   - Interictal spikes or sharp waves suggest epilepsy.
3. Neuroimaging
   - **MRI:** To identify structural abnormalities (*e.g.*, tumors, cortical dysplasia).
   - **CT Scan:** Used in emergencies to detect acute lesions.
4. Blood Tests
   - Identify metabolic or infectious causes.
5. Genetic Testing
   - Performed in cases of suspected genetic epilepsies

PRINCIPLE

In experimental animals, epilepsy of various types, including grand mal, petit mal, and psychomotor type, can be investigated. Anticonvulsant medications are tested in laboratory animals using two ways to examine convulsion: chemo-convulsion caused by pentylenetetrazol, which causes clonic type convulsion in humans, and Maximum Electroshock (MES) generated convulsions in animals that simulate grandmal epilepsy [1]. Convulsion electroshock is administered *via* the ocular electrodes in MES. Cortical excitation is created through ocular stimulation [2]. The MES convulsion occurs in five stages.

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

It is advised that students have a solid understanding of the pharmacology of anti-epileptic drugs before carrying out this experiment. A substance is deemed to have anti-convulsant qualities if it lowers or eliminates the extensor phase of MES convulsions in both rats and mice.

REQUIREMENT

Animal: Rats weighing between 150 and 200 grams.

Drugs: Make a stock solution and administer 25 mg/kg of phenytoin.

Equipment: An electroconvulsimeter, a cornel electrode (150 mA current for 0.2 sec), and a stopwatch.

PROCEDURE

1. Weigh and number the animals. Divide them into two groups, each with four or five rats. One group receives drug treatment, while the other serves as the control.
2. Hold the animals securely, and then apply the recommended current while placing corneal electrodes on the cornea. Observe the various convulsion stages.
3. Replicate with additional members of the control group.
4. Inject a batch of four to five rats with phenytoin I.P. After 30 minutes, the animals were made to undergo electroconvulsions.
5. MES convulsions show a reduction in duration or elimination of the tonic extensor [3].

Table 7. Observation of seizure phases and recovery in rodents following seizure induction.

S. No.	Onset time (sec)	Tonic limb flexor (sec)	Tonic extensor (sec)	clonus (sec)	Stupor (sec)	Recovery (sec)	Non-Extensor Seizure
1.	35	5	10	20	60	120	Yes
2.	40	6	12	18	55	115	No
3.	38	7	11	22	62	130	Yes
4.	42	5	13	19	58	125	No

CHAPTER 13

Drugs Used for Anti-Cationic Activity and Stereotype-Like Behavior in Murinea

OBJECTIVES

1. To study drug (phenothiazines) induced catatonia (extrapyramidal side effect in rats).
2. To study the anticatatonic (antiparkinsonian) effect of scopolamine.

PRINCIPLE

It is known that antipsychotic medications of the phenothiazine and butyrophenone types cause extrapyramidal adverse effects in humans. These side effects, which include tremors, rigidity, and akinesia, are referred to as Parkinson-like since one of the main clinical symptoms of Parkinson's disease is difficulty moving. The side effects of antipsychotic medication are brought on by excessive blocking of the extrapyramidal motor system's dopamine receptors. Consequently, phenothiazines (perphenazine or chlorpromazine) are frequently used to cause extrapyramidal symptoms similar to Parkinson's symptoms in lab animals and to research medications that treat Parkinson's disease. Students are recommended to be familiar with the pharmacology of anti-Parkinsonian medications before conducting this experiment [1, 2].

REQUIREMENTS

Animal: Rats (150-200 g)

Drugs:

- Perphenazine (dose: 5 mg/kg i.p.; make a stock solution with 1 mg/ml of the medication, and then inject 0.5 ml per 100 g of the animal's body weight).
- Scopolamine (dosage: 2 mg/kg; inject 0.5 ml/100 g of the animal's body weight after making a stock solution containing 0.4 mg/ml of the medication). Equipment: Two wooden blocks, one measuring three centimeters in height and the other measuring nine centimeters.

Cationic Activity of Drugs

Cationic activity refers to the ability of drugs to carry a positive charge and interact with negatively charged biological molecules or structures such as cell membranes, DNA, RNA, or proteins. This property is particularly important in certain drug classes, as it significantly influences their mechanism of action, pharmacodynamics, and therapeutic applications.

Mechanism of Cationic Activity

Drugs with cationic properties interact with negatively charged components through electrostatic interactions. For example:

1. **Cell Membranes**: Many bacterial membranes have a negative charge due to phospholipids and lipopolysaccharides. Cationic drugs bind to these sites, disrupting membrane integrity, leading to cell lysis or altered permeability.
2. **Nucleic Acids**: Some cationic drugs interact with negatively charged DNA or RNA, inhibiting replication, transcription, or translation.
3. **Protein Targets**: Cationic drugs may also bind to negatively charged functional groups on enzymes or structural proteins, affecting their activity.

Examples of Drugs with Cationic Activity

1. **Aminoglycosides**:

- Bind to the negatively charged 16S ribosomal RNA in the bacterial 30S subunit, disrupting protein synthesis.

2. **Quaternary Ammonium Compounds**:

- Act as disinfectants by disrupting bacterial and fungal cell membranes.

3. **Antimalarial Drugs (*e.g.*, Chloroquine)**:

- Bind to negatively charged heme in the parasite's food vacuole, preventing detoxification and leading to parasite death.

4. **Polymyxins (*e.g.*, Polymyxin B, Colistin)**:

- Interact with the negatively charged phosphate groups in the bacterial outer membrane, disrupting its structure and function.

Significance of Cationic Activity

- **Antimicrobial Action**: Many antibiotics and antiseptics rely on cationic activity

to target pathogens selectively.

- **Target Specificity**: The electrostatic interactions enhance the drug's ability to selectively bind to microbial or diseased tissues.
- **Drug Delivery**: Cationic nanoparticles or liposomes are used to enhance drug delivery by interacting with negatively charged cell surfaces or DNA.

PROCEDURE

1. Weigh and number the animal. Divide the animals into two groups: one for studying the scopolamine effect and another for assessing the perphenazine impact (control group). Each group should contain a minimum of five animals (Tables **10** & **11**).
2. Use perphenazine injections to manage animals. Note the catatonic response's severity.
3. After taking perphenazine, note the degree of catatonia at 5, 15, 30, 45, 60, 90, and 120 minutes.
4. The animals in the second group were given scopolamine injections, followed by a 30-minute injection of perphenazine. Assess the degree of catatonia by observing and scoring as in step 2.
5. Examine the difference in the two groups' catatonic responses' beginning and intensity. Draw a graph with time plotted on the x-axis. Keep in mind that there were differences in the onset and intensity of catatonic reactions in both groups [3 - 6].

Table 10. Effect of Perphenazine (5 mg/kg, I.P) on Catatonia Induction in Mice at Different Time Intervals.

S. No.	Weight of Animal	Dose	Dose to be Administered	Mean Catatonia After Minutes of PERP Treatment					
			Perphenazine (mg)	15	30	45	60	90	120
1.	180	Perphenazine (5mg/kg)	0.9	**0.5**	**1.0**	**2.5**	**3.5**	**3.0**	**1.5**
2.	185		0.92	**0.5**	**1.5**	**2.8**	**3.8**	**3.2**	**1.8**
3.	205		1.02	**0.8**	**1.2**	**3.0**	**4.0**	**3.3**	**1.7**
4.	210		1.05	**0.7**	**1.4**	**2.9**	**4.2**	**3.5**	**1.6**
5.	200		1	**0.6**	**1.3**	**2.7**	**3.9**	**3.1**	**1.5**
	Mean		**0.798**	**0.62**	**1.28**	**2.78**	**3.8**	**3.2**	**1.6**

CHAPTER 14

Study of Anxiolytic Activity of Drugs Using the Murine

OBJECTIVE

To studies the Anxiolytic Activity of Drugs Using Murines.

Anxiety

Anxiety is a natural response to perceived threats. It involves a heightened state of alertness that is accompanied by physiological changes, such as an increased heart rate and muscle tension. This response, often referred to as the "fight or flight" mechanism, has evolutionary roots and is meant to help individuals deal with danger.

However, modern stressors, such as work pressure, financial difficulties, or personal relationships, can trigger this response unnecessarily, leading to chronic anxiety in some individuals [1].

Symptoms of Anxiety

Emotional Symptoms

1. Excessive worry or fear.
2. Feelings of restlessness or being "on edge."
3. Irritability or frustration.
4. A sense of impending doom or danger.

Physical Symptoms

1. Rapid heartbeat (tachycardia).
2. Shortness of breath or hyperventilation.
3. Sweating or chills.
4. Trembling or shaking.
5. Muscle tension or fatigue.
6. Nausea, dizziness, or gastrointestinal disturbances.
7. Difficulty sleeping (insomnia).

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Cognitive Symptoms

1. Trouble concentrating or staying focused.
2. Overthinking or racing thoughts.
3. Catastrophizing (assuming the worst will happen).
4. Indecisiveness or feeling overwhelmed.

Causes of Anxiety

Biological Factors

1. **Neurotransmitter Imbalance**: Abnormal levels of serotonin, dopamine, and Gamma-Aminobutyric Acid (GABA) can contribute to anxiety.
2. **Genetics**: A family history of anxiety disorders increases the risk.
3. **Brain Structure**: Hyperactivity in the amygdala, which processes fear, may play a role.

Psychological Factors

1. **Past Trauma**: Experiencing traumatic events can increase susceptibility to anxiety.
2. **Learned Behavior**: Growing up in an environment of fear or excessive worry may predispose individuals to anxiety.

Environmental Factors

1. **Stressful Life Events**: Financial difficulties, relationship problems, or academic pressures.
2. **Substance Abuse**: Alcohol, caffeine, or drugs can exacerbate anxiety.
3. **Chronic Illness**: Conditions like diabetes, cardiovascular disease, or chronic pain can contribute to anxiety.

Types of Anxiety Disorders

1. Generalized Anxiety Disorder (GAD):

- Persistent and excessive worry about everyday matters.
- Symptoms last for at least six months.

2. Panic Disorder:

- Characterized by sudden and intense episodes of fear (panic attacks).
- Symptoms include chest pain, palpitations, and feelings of losing control.

3. Social Anxiety Disorder (SAD):

- Intense fear of being judged or embarrassed in social situations.
- Can lead to avoidance of social interactions.

4. Specific Phobias:

- Extreme fear of a particular object or situation (*e.g*., heights, spiders).
- The fear is disproportionate to the actual danger.

5. Obsessive-Compulsive Disorder (OCD):

- Characterized by intrusive thoughts (obsessions) and repetitive behaviors (compulsions).

6. Post-Traumatic Stress Disorder (PTSD):

- Develops after exposure to a traumatic event.
- Symptoms include flashbacks, nightmares, and heightened arousal.

7. Separation Anxiety Disorder:

- Fear of being separated from attachment figures, more common in childrenmbut can affect adults.

PRINCIPLE

Anxiety is characterized as a state of unease, ambiguity, and strain resulting from the expectation of a perceived or hypothetical danger. The elevated zero-maze test is a behavioral anxiety test that uses rats' naturalistic avoidance behavior as its basis in places that are open and lofty. It resembles the elevated plus maze, which is more popular, and the closed arms are positioned in a circle, removing the middle portion, which eliminates the unclear interpretation of the amount of time spent in the traditional central square style. An elevated (40-centimeter) white or black circular maze with an inner diameter of 30 cm and an outside diameter of 45 cm is what it looks like. The mouse can explore a 6 cm wide runway ring that is separated into four quadrants, with two “open” wall-free quadrants and two “closed” quadrants with walls that are 12 cm high. A 2-3 mm ridge surrounds the open quadrants to keep mice from falling off the walls. The thickness is 0.75 cm [2].

REQUIREMENTS

Animal: Mice

CHAPTER 15

Study of Local Anaesthetics by Different Methods

OBJECTIVE

To study the effect of local anesthetics by different methods.

PRINCIPLE

Local anesthetics impede impulse conduction *via* excitable membranes and nerve axons in a reversible manner. They act to inhibit pain perception and to create local or regional anesthesia. It is simple to study the local anesthetic property by employing any of the **3** ***below mentioned*** techniques, namely; (a) nerve block anesthesia (Solman method), where the medication is administered in proximity to the nerve trunk; (b) surface anesthesia, where the medication is injected into the eye's conjunctiva and corneal reflux is observed toward a pointed object and (c) anesthesia infiltration (in which the medication is injected intradermally and the location is examined for pinprick sensitivity).

Local Anesthetics

Local anesthetics [1] are a class of drugs that temporarily block the transmission of nerve impulses, leading to a reversible loss of sensation in a specific area of the body. Unlike general anesthetics, local anesthetics do not cause loss of consciousness. They are widely used in medical and dental procedures to manage pain and facilitate minor surgeries, diagnostic procedures, and other interventions.

Mechanism of Action

Local anesthetics work by inhibiting the conduction of electrical impulses in nerve fibers [2]. This is achieved by:

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1. **Targeting Sodium Channels**:
   - Local anesthetics bind to voltage-gated sodium channels in the nerve membrane.
   - This binding inhibits the influx of sodium ions, which is essential for the depolarization phase of the action potential.
2. **Blocking Nerve Conduction**:
   - When sodium channels are blocked, the nerve cannot generate or propagatemaction potentials.
   - This prevents the brain from receiving pain signals from the affected area.

Types of Local Anesthetics [3]

Based on Chemical Structure

1. **Ester-linked Anesthetics**:
   - Shorter duration of action due to rapid metabolism by plasma esterases.
   - Examples: Procaine, Chloroprocaine, Benzocaine.
2. **Amide-linked Anesthetics**:
   - Longer duration of action and slower metabolism by liver enzymes.
   - Examples: Lidocaine, Bupivacaine, Ropivacaine, Mepivacaine.

Based on Duration of Action

1. **Short-Acting**:
   - Examples: Procaine, Chloroprocaine.
2. **Intermediate-Acting**:
   - Examples: Lidocaine, Mepivacaine.
3. **Long-Acting**:
   - Examples: Bupivacaine, Ropivacaine.

Clinical Uses

1. **Topical Anesthesia**:
   - Applied directly to mucous membranes or skin.
   - Examples: Benzocaine, Lidocaine (in creams, sprays, gels).
2. **Infiltration Anesthesia**:
   - Injected into tissues for minor surgical procedures or wound suturing.
   - Examples: Lidocaine, Procaine.
3. **Nerve Block Anesthesia**:
   - Injected near a specific nerve or nerve plexus to block sensation in a larger area.
   - Examples: Bupivacaine, Ropivacaine.

4. **Spinal Anesthesia**:
   - Injected into the subarachnoid space to anesthetize lower body regions.
   - Examples: Lidocaine, Tetracaine.
5. **Epidural Anesthesia**:
   - Injected into the epidural space, commonly used in childbirth and surgeries.
   - Examples: Bupivacaine, Ropivacaine.
6. **Intravenous Regional Anesthesia (Bier Block)**:
   - An anesthetic is injected into a vein of an extremity, often combined with a tourniquet.
   - Example: Lidocaine.

Adverse Effects

While local anesthetics are generally safe when used correctly, adverse effects may occur due to systemic absorption, overdose, or allergic reactions.

1. **Central Nervous System (CNS)**:
   - Early symptoms: Restlessness, dizziness, tinnitus, metallic taste.
   - Severe symptoms: Seizures, respiratory depression, or coma.
2. **Cardiovascular System**:
   - Bradycardia, hypotension, or arrhythmias.
   - Bupivacaine, in particular, is associated with cardiotoxicity.
3. **Allergic Reactions**:
   - More common with ester-linked anesthetics due to the formation of Para-Aminobenzoic Acid (PABA), a known allergen.
4. **Local Tissue Toxicity**:
   - Prolonged use can cause nerve damage or tissue necrosis.

Contraindications

1. **Severe liver disease**: Amide anesthetics are metabolized in the liver and may accumulate.
2. **Allergy to local anesthetics**: Particularly ester-linked anesthetics.
3. **Heart conditions**: Use with caution in patients with arrhythmias or severe bradycardia.

REQUIREMENTS

Animal: frog

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

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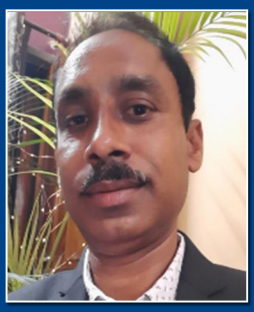

Kedar Prasad Meena

Dr. Kedar Prasad Meena Associate Professor (Pharmaceutics) Department of Pharmacy Guru Ghasidas Central Vishwavidyalaya Bilaspur C.G. 495009, M-9424160514, Ph.D A Central University, Guru Ghasidas Vishwavidyalaya, Bilaspur, CG. Master of Pharmacy from BIT, Mesra Ranchi University, JH. Bachelor of Pharmacy from Rajasthan University, Jaipur Teaching & Research Experience 22 years. He published 34 research papers in various national and international journals. He has attended many national and international conferences, seminars, webinars, FDP, and workshops and Life Member of the Association of Pharmaceutical Teachers of India (APTI).

Suman Kamlesh Rattan

Ms. Suman Kamlesh Rattan, a Ph.D. Scholar in Pharmacology under the supervision of Dr. Kedar Prasad Meena, Associate Professor of Pharmaceutics at Guru Ghasidas Vishwavidyalaya (GGV), Bilaspur, Chhattisgarh. Ms. Rattan has completed a Bachelor of Pharmacy from Department of Pharmacy, Pacific University Udaipur and Master of Pharmacy in Pharmacology from IK. Gujral Punjab Technical University, Kapurthala, Jalandhar and has 1 publication to her credit and attended many National & International conferences, Seminars, webinars and workshops.

Venu Anand Das Vaishnav

Mr. Venu Anand Das Vaishnav, a Ph.D. Scholar in Pharmacology under the supervision of Dr. Nishant Sudhir Jain, Associate Professor of Pharmacology at Guru Ghasidas Vishwavidyalaya (GGV), Bilaspur, Chhattisgarh. Mr. Vaishnav has completed a Bachelor of Pharmacy from Royal College of Pharmacy, Raipur, Chhattisgarh, and a Master of Pharmacy in Pharmacology from Guru Ghasidas Vishwavidyalaya, Bilaspur, Chhattisgarh. He has published 2 research articles and 4 review articles, along with contributing to multiple book chapters. He has participated in numerous national and international conferences, seminars, and workshops and was awarded the Best Oral Presentation Award in 2024 for his research work.