3rd ANNUAL WORKSHOP

on Animal Handling & Experimental Pharmacology

Organized By
Department of Pharmacology, IGIMS Patna

Under Aegis of
Academic Society of Pharmacologist of IGIMS (ASPIGIMS)

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SOUVENIR cum Abstract Book

3rd Annual Workshop on Animal Handling & Experimental Pharmacology

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Supported By:

Date : 17th - 18th October 2025

Venue: Department of Pharmacology, 3rd Floor, IGIMS, Patna - 800 014

Message

Prof. (Dr.) Bindey Kumar
Director-cum- Vice Chancellor
IGIMS, Patna
Patron in Chief

It gives me immense pleasure to extend my greetings for the **'3rd Annual Workshop on Animal Handling & Experimental Pharmacology'.** The resounding success of the previous workshops has solidified this event as a cornerstone in the academic calendar of our institution.

This workshop embodies our unwavering commitment to bridging the gap between theoretical knowledge and practical expertise. In an era of rapid advancements in biomedical research, a strong foundation in experimental pharmacology is not just an asset but a necessity. This event is meticulously designed to empower our future scientists and clinicians with the skills to conduct rigorous, ethical, and impactful research.

I commend the Department of Pharmacology for their relentless efforts in organizing this pivotal event and for fostering a culture of excellence and ethical responsibility. I am confident that this workshop will significantly contribute to the professional growth of all participants and advance the frontiers of preclinical research.

Message

Prof. (Dr.) Harihar Dikshit
Prof & Head, Department of Pharmacology
Cum
Principal
Netaji Subhas Medical College & Hospital, Bihta, Patna, Bihar
Professor Emeritus, Dept of Pharmacology, IGIMS, Patna,
President, ASPIGIMS Society,
Patron

As President of the Academic Society of Pharmacologists of IGIMS, it is heartening to see the continued growth and impact of this annual workshop. Our journey to revive robust practical training in experimental pharmacology is gaining momentum, thanks to the collective efforts of our faculty and the establishment of our state-of-the-art Central Animal House facility.

This year's workshop continues to address a vital need, equipping our postgraduate and PhD scholars with the indispensable tools for their research careers. The comprehensive curriculum, which spans from CCSEA compliance to sophisticated bioassay techniques, is designed to create well-rounded, ethical, and skilled researchers.

I extend my gratitude to all the national and international faculties for sharing their expertise. Let us continue to champion high standards in education and research. I wish all participants a successful learning experience.

Message

Prof. (Dr.) Om Kumar
MD, DM ,DNB(Nephro)
Dean (Academics)&
HOD, Dept. of Nephrology,
IGIMS, Patna

I am delighted to welcome all participants, faculty, and guests to the **3rd Annual Workshop on Animal Handling & Experimental Pharmacology**. This workshop represents a critical initiative in our ongoing mission to provide a comprehensive and modern medical education.

The hands-on skills imparted here—from ethical guidelines and animal handling to advanced screening techniques—are fundamental to the development of a competent medical researcher. This workshop provides a unique platform for students and professionals to learn from leading experts and gain proficiency in the core methodologies that drive pharmacological discovery.

I encourage each participant to engage actively in the sessions, ask questions, and collaborate with peers. The knowledge and networks you build here will be invaluable. My best wishes for a productive and enlightening workshop.

Message

Prof. (Dr.) Bibhuti P. Sinha
Patron, Deputy Director Academics,
HOD, Regional Institute of Ophthalmology,
IGIMS, Patna

It is a privilege to associate myself with the 3rd Annual Workshop on Animal Handling & Experimental Pharmacology. This event stands as a beacon of our institute's dedication to fostering academic excellence and innovative research.

The ethical and humane use of animals in research is a responsibility we all share. This workshop not only emphasizes the 'how' of experimental techniques but, just as importantly, the 'why' behind ethical principles and regulatory frameworks. This balanced approach is essential for conducting credible and conscientious science.

I applaud the organizers for assembling a remarkable program and distinguished resource persons. I am confident that the discussions and demonstrations will inspire new ideas and refine existing practices, contributing significantly to the field. Wishing you all a fruitful workshop.

Message

Prof. (Dr.) Lalit Mohan
Associate Dean (Exam),
Prof & HOD, Dept. of Pharmacology, IGIMS, Patna
Vice- President, ASPIGIMS
Organizing Committee Chairman

With great pride and a deep sense of accomplishment, I extend a warm welcome to all participants of the **3rd Annual Workshop on Animal Handling & Experimental Pharmacology**, organized under the aegis of the *Academic Society of Pharmacologists of IGIMS (ASPIGIMS)*. Building on the strong foundation laid by our previous workshops, this year's program continues our mission of providing a **comprehensive and practice-oriented learning experience**—covering every facet of animal experimentation, from the fundamentals of rodent biology and drug administration to advanced pharmacological screening, regulatory guidelines, and data interpretation.

This workshop would not have been possible without the vision and encouragement of our esteemed **Patrons**:

- **Prof. (Dr.) Bindey Kumar**, Director, IGIMS, Patna
- **Prof. (Dr.) Om Kumar**, Dean, IGIMS, Patna
- **Prof. (Dr.) B. P. Sinha**, Deputy Director, IGIMS, Patna
- **Prof. (Dr.) Harihar Dikshit**, Professor Emeritus, Department of Pharmacology, IGIMS, Patna

I express my heartfelt gratitude to each of them for their constant support and guidance.

I also extend my sincere thanks to our distinguished **resource faculty** who have joined us from across the country to share their expertise:

- **Prof. (Dr.) N. R. Biswas**, Vice Chancellor, Sri Balaji Vidyapeeth, Pondicherry
- **Prof. (Dr.) Bikash Medhi**, PGIMER, Chandigarh
- **Dr. U. S. Chakradhara Rao**, JIPMER, Pondicherry
- **Dr. Vinod Nair**, Director, Oncology & Precision Medicine, Gurugram

- **Dr. Pramod Kumar Majhi**, HOD, Dept. of Pharmacology, AIIMS, Patna
- Along with our dedicated colleagues and young researchers from IGIMS, AIIMS Patna, NIPER Hajipur, NSMCH Bihta, and other institutions, who will be leading the hands-on demonstrations and training sessions.

I would also like to thank MYCALPHARM software for providing us platform to demonstrate complex experimental procedure minimizing un-necessary exploitation of animals for demonstration purposes.

Your presence and contributions enrich this workshop and inspire our postgraduate students to pursue research with both **scientific rigor and ethical responsibility**.

I must also acknowledge the tireless efforts of the **Organizing Secretary, Prof. (Dr.) Manish Kumar**, and the entire organizing team, whose meticulous planning and commitment have shaped this event into a vibrant academic platform.

It gives me immense pleasure to also announce the **publication of the first edition of the book**: **"Preparatory Manual of Animal Handling & Experimental Pharmacology: For Postgraduate Pharmacology Students."** This manual is designed to serve as a practical guide and reference resource, bridging theoretical knowledge with experimental skills, and will undoubtedly support the next generation of pharmacologists in their academic and research pursuits.

Finally, I extend my heartfelt appreciation to all participants. Your enthusiasm, curiosity, and active engagement are the true driving forces behind this initiative. Together, let us make this workshop not only a success but also a meaningful step forward in advancing the science of pharmacology and experimental medicine.

Message

Prof. (Dr.) Manish Kumar,
Professor,
Dept. of Pharmacology, IGIMS, Patna,
General Secretary, ASPIGIMS,
Organizing Secretary

It is with great enthusiasm that I welcome you to the "3rd Annual Workshop on Animal Handling & Experimental Pharmacology." As the Organizing Secretary, it is both an honor and a responsibility to present a program that meets the highest standards of educational and practical value.

This workshop has been structured to be a complete journey through the world of experimental pharmacology. We will begin with the essential regulatory framework of the CCSEA and Form B, proceed to core practical skills in animal handling and drug administration, and advance to demonstrations of screening for various pharmacological activities and bioassays. The inclusion of modern tools like CAL and the semi-auto analyzer ensures that our participants are industry-ready.

I invite you to immerse yourselves fully in this learning experience. My sincere thanks to all the collaborators, faculty, and team members. I am confident that this workshop will be an invaluable milestone in your professional journey.

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Prof. (Dr.) Bindey Kumar
Director-cum-Vice Chancellor
IGIMS, Patna

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Prof. (Dr.) N R Biswas
Vice Chancellor, Sri Balaji Vidyapeth
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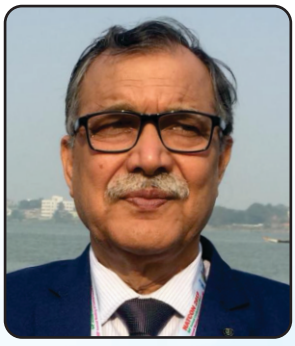

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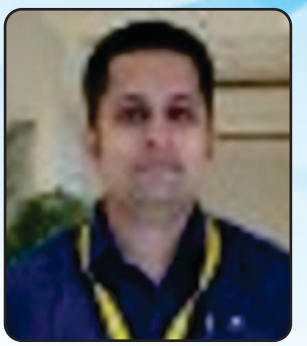

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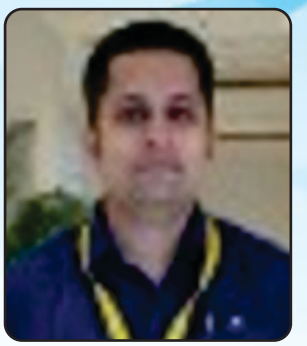

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Workshop Program Schedule

3rd Annual Workshop on

"Animal Handling & Experimental Pharmacology"

Organized by the Department of Pharmacology, IGIMS, Patna

Under the aegis of "Academic Society of Pharmacologist of Indira Gandhi Institute of Medical Sciences" (ASPIGIMS)

Date: 17-10-2025 & 18-10-2025

Venue: Department of Pharmacology & LT 3 & 4, Medical College Building, IGIMS, Patna-14

Day 1: 17-10-2025		
TIME	**SESSIONS**	**RESOURCE PERSONS**
8:30-9:00 AM	**Registration & Breakfast (Lecture Theatre 3 & 4 - LT3,4, 2nd Floor, Medical College Building, IGIMS)**	
09:00-09:20 AM	**Inaugural Lecture:** Current and Future Perspectives of Animal Research in Drug Discovery	**Dr. U. S. Chakradhara Rao**, Dept. of Medical Oncology, JIPMER, Pondicherry
09:20-10:00 AM	Committee for Control and Supervision of Experiments on Animals (CCSEA): Rules & Guidelines & Hands on training to fill the form B (IAEC)	**Dr. Manoj Kumar**, Veterinary Officer, Dept. of Pharmacology, IGIMS, Patna **Dr. Manish Kumar**, Professor, Pharmacology, IGIMS, Patna **Dr. Pankaj Prabhakar**, Scientist I, Pharmacology, IGIMS, Patna
10:00-10:20 AM	**Inauguration of Workshop, Book Release & Welcome note** **Patron in Chief:** **Prof. (Dr.) Bindey Kumar**, Director, IGIMS, Patna **Patron:** **Prof. (Dr.) Om Kumar**, Dean, IGIMS, Patna **Patron:** **Prof. (Dr.) B. P. Sinha,** Deputy Director, IGIMS, Patna **Patron:** **Prof. (Dr.) Harihar Dikshit**, Prof. Emeritus, Dept. of Pharmacology, IGIMS, Patna **Organizing Chairman:** **Prof. (Dr.) Lalit Mohan**, Prof. & Head, Dept. of Pharmacology, IGIMS, Patna **Organizing Secretary:** **Prof. (Dr.) Manish Kumar**, Prof, Dept. of Pharmacology, IGIMS, Patna	
10:20-11:20 AM Venue: Lecture theatre 3, 2nd Floor, Medical College Building, IGIMS, Patna	**Panel Discussion:** **From Bench to Bedside Without Animals: Myth or Reality?**	**Moderator:** **Prof. (Dr.) Harihar Dikshit,** Principal & HOD, Dept. of Pharmacology, NSMCH, Bihta **Panelists:** **Prof. (Dr.) N R Biswas**, Vice Chancellor, Sri Balaji Vidyapeeth, Pondicherry **Prof. (Dr.) Bikash Medhi**, Professor, Dept. of Pharmacology, PGIMER, Chandigarh **Dr. U. S. Chakradhara Rao**, Dept. of Medical Oncology, JIPMER, Pondicherry **Dr. Vinod Nair,** Director, Oncology & Precision Medicine, Z S Associates, Gurugram, Haryana
11:20-11:30 AM	**Break**	
11:30 AM – 1:30 PM (Participants will be divided into four groups, and each group will rotate to one of the four stations every 30 minutes) Venue: Dept. of Pharmacology, 3rd Floor, Medical College Building, IGIMS	Rodent biology and Gender identification of laboratory animals, identification of species and strain Identification of animal behavior and common, signs of illness	**Dr. Manoj Kumar**, Veterinary Officer, Dept. of Pharmacology, IGIMS, Patna **Dr. Bazla Nazir**, PG Student, Dept. of Pharmacology, IGIMS, Patna
	Handling of Rodents (Mice and Rats), Rabbits, Guinea pigs Demonstration of techniques and instruments for oral feeding (gavage); subcutaneous, intramuscular, and intra- peritoneal administration Demonstration of techniques of collection of blood from tail vein of rats, orbital plexus of rats	**Dr. Pankaj Prabhakar**, Scientist I, Dept. of Pharmacology, IGIMS, Patna **Dr. Amrendra Kumar Arya,** Senior Resident, Pharmacology, **Dr. Ruhi Bhimseria**, PG Student, Dept. of Pharmacology, IGIMS, Patna
	Demonstration of Screening of Anti-inflammatory activity	**Dr. Arka Mondal**, Assistant Prof., SGT Medical College, Haryana **Dr. Trisha Priyadarshini**, PG Student, Dept. of Pharmacology, IGIMS, Patna
	Demonstration of Screening of Effect of Drugs on Wound Healing	**Dr. Nitesh Kumar**, Assistant Professor, Dept. of Pharmacology & Toxicology, NIPER, Hajipur & **Dr. Bhawana A. Verma**, PG Student, Dept. of Pharmacology, IGIMS, Patna

Workshop Program Schedule

3rd Annual Workshop on

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Organized by the Department of Pharmacology, IGIMS, Patna
Under the aegis of "Academic Society of Pharmacologist of Indira Gandhi Institute of Medical Sciences" (ASPIGIMS)
Date: 17-10-2025 & 18-10-2025
Venue: Department of Pharmacology & LT 3 & 4, Medical College Building, IGIMS, Patna-14

1:30-2:30 PM	**LUNCH (Dept. of Pharmacology, 3rd Floor, Medical College Building, IGIMS, Patna)**	
2:30-04:30 PM (Participants will be divided into four groups, and each group will rotate to one of the four stations every 30 minutes) Venue: Department of Pharmacology, 3rd Floor, Medical College Building, IGIMS, Patna	Demonstration of Screening of Antiepileptic Activity	**Dr. Sunil Kumar Singh**, Associate Prof., Dept. of Pharmacology, AIIMS, Patna **Dr. Ravi Roushan**, SR, Dept. of Pharmacology, IGIMS, Patna,
	Demonstration of Screening of Analgesic Activity	**Dr. Amritanshu Shekhar**, Assistant Professor, Dept. of Pharmacology, NSMCH, Bihta **Dr. Pallavi Kumari**, DM Student **Dr. Chandani Prakash**, PG Student, Dept. of Pharmacology, IGIMS, Patna
	Demonstration of screening of Anti-anxiety Activity	**Prof. (Dr.) Lalit Mohan**, Prof. & Head, Dept. of Pharmacology, IGIMS, Patna **Dr. Mukesh Kumar**, CMO cum SR, Dept. of Pharmacology, ESICMCH, Bihta, Patna **Dr. Syed Sharjil Anees**, DM Student, Dept. of Pharmacology, IGIMS, Patna
	Demonstration of Screening of Effect of Drugs on Neuromuscular Coordination	**Prof. (Dr.) Manish Kumar**, Professor, Dept. of Physiology, IGIMS, Patna **Dr. Rajeev Kumar Neeraj**, PG Student, Pharmacology, **Dr. Purnendu Arya,** Ex-Senior Resident, Dept. of Pharmacology, IGIMS, Patna
04:30-05:30 PM	**Hi-Tea** **Poster/ Oral Presentation**	**Dept. of Pharmacology, 3rd Floor, Medical College Building, IGIMS, Patna**

Day 2: 18-10-2025

TIME	SESSIONS	RESOURCE PERSONS
8:30-9:00 AM	**Breakfast (Lecture Theatre 3 & 4 - LT3,4, 2nd Floor, Medical College Building IGIMS, Patna)**	
9:00-9:30 AM	Regulatory toxicities studies for IND applications	**Prof. (Dr.) Bikash Medhi**, Professor, Dept. of Pharmacology, PGIMER, Chandigarh
9:30-10:00 AM	How to calculate LD_{50}	**Dr. Pramod Kumar Majhi**, Associate Prof., HOD, Dept. of Pharmacology, AIIMS, Patna
10:00-10:30 AM	ARRIVE Guidelines	**Dr. Ramesh Kumar Nirala**, Associate Prof., Dept. of Veterinary Pharmacology & Toxicology, Bihar Veterinary College, Patna
10:30-11:00 AM	MyCal Pharm Software	**Mrs. Kunjan Arora,** Director, Infokart India Pvt. Ltd., New Delhi, India
11:00 – 11:30 AM	**Hi- Tea (Dept. of Pharmacology, 3rd Floor, Medical College Building, IGIMS, Patna)**	
11:30 AM – 1:30 PM (Participants will be divided into four groups, and each group will rotate to one of the four stations every 45 minutes) Venue: Department of Pharmacology, 3rd Floor, Medical College Building, IGIMS, Patna	Demonstration of Experimental Set- Up for Bioassay & Calculation from Physical Graph	**Dr. Sukalyan Saha Roy**, Addl Prof., Dept. of Pharmacology, IGIMS, Patna **Dr. Nidhi Kumari**, Senior Resident, Pharmacology, IGIMS,
	Demonstration of Bioassay of Histamine by Three Point Assay Method and Calculation of PD2 & PA2 using Computer Assisted Learning (CAL)	**Dr. Adil Ali Shakur**, Associate Prof., Dept. of Pharmacology, IGIMS, Patna **Dr. Saajid Hameed**, SR, Dept. of Pharmacology, IGIMS, Patna **Dr. Ravi Roushan**, SR, Dept. of Pharmacology, IGIMS, Patna
	Demonstration of effects of drugs on HR & BP of Dog using Computer Assisted Learning (CAL)	**Prof. (Dr.) Hitesh Mishra**, Prof., Dept. of Pharmacology, IGIMS, Patna **Dr. Raushan K. Ranjan**, Ex-Senior Resident, IGIMS, Patna **Dr. Nishi**, DM Student & Dept. of Pharmacology, IGIMS, Patna
	Demonstration of Soxhlet and other related apparatuses	**Dr. Manish Kumar**, Prof., Dept. of Pharmacology, IGIMS, Patna **Dr. Keshav Kumar**, DM Student, Dept. of Pharmacology, IGIMS, Patna
01:30-02:30 PM	**Lunch (Dept. of Pharmacology, 3rd Floor, Medical College Building, IGIMS, Patna)**	
02:30-03:00 PM	**Concluding Remarks, Valedictory Function & Certificate Distribution** **Venue: Lecture theatre 3, 2nd Floor, Medical College Building, IGIMS, Patna**	

Summary of Presentations of Resource Faculties

CURRENT AND FUTURE PROSPECTIVE OF ANIMAL RESEARCH IN DRUG DISCOVERY

Dr. U S Chakradhara Rao, Dept. of Medical Oncology, JIPMER, Pondicherry

The field of drug discovery has undergone significant transformations over the past few decades, driven by advancements in technology, a deeper understanding of biological systems, and evolving regulatory landscapes. Among the various methodologies employed in drug discovery, animal research continues to play a pivotal role. This essay explores the current state of animal research in drug discovery, its ethical implications, the challenges it faces, and the future prospects that may reshape its application in the pharmaceutical industry.

Current State of Animal Research in Drug Discovery

Animal research has been a cornerstone of biomedical science for centuries, providing critical insights into human physiology and disease mechanisms. In the context of drug discovery, animal models are invaluable for evaluating the efficacy and safety of new therapeutic agents before they are tested in humans. These models allow researchers to study complex biological interactions and disease processes that cannot be effectively replicated in vitro.

Currently, several animal species are commonly used in drug discovery, including mice, rats, rabbits, dogs, and non-human primates. Mice, in particular, are the most widely used due to their genetic similarities to humans, short reproductive cycles, and well-characterized genomes. Animal studies are essential for assessing pharmacokinetics (how a drug is absorbed, distributed, metabolized, and excreted), pharmacodynamics (the effects of the drug on the body), and potential side effects. Regulatory agencies, such as the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA), require animal testing as part of the preclinical evaluation process for new drugs.

Despite the essential role of animal research, it is not without its challenges and controversies. Ethical concerns regarding the welfare of animals used in research have led to increased scrutiny and calls for the adoption of the "3Rs" principle: Replacement, Reduction, and Refinement. Researchers are encouraged to replace animal models with alternative methods wherever possible, reduce the number of animals used in experiments, and refine experimental procedures to minimize suffering.

Challenges Facing Animal Research

Despite its critical role in drug discovery, animal research faces several challenges. One significant issue is the translational gap between animal models and human outcomes. Many drugs that show promise in animal studies fail to demonstrate efficacy or safety in human clinical trials. This discrepancy can be attributed to differences in physiology, genetics, and disease expression between humans and animal models. Consequently, there is a growing demand for the development of more predictive models that can better mimic human conditions.

Another challenge is the increasing pressure from regulatory bodies and the public to reduce reliance on animal testing. As scientific advancements continue to evolve, alternative methods such as in vitro testing, computer modeling, and organ-on-a-chip technologies are being explored. These approaches offer the potential to reduce the number of animals used in research while still providing valuable data on drug interactions and effects.

Future Prospects of Animal Research in Drug Discovery

Looking ahead, the future of animal research in drug discovery is likely to be shaped by several key trends and innovations. One promising direction is the integration of advanced technologies such as genomics, proteomics, and bioinformatics into animal research. These technologies can enhance our understanding of disease mechanisms and drug responses, potentially leading to the development of more relevant animal models.

Furthermore, the emergence of humanized animal models—animals genetically modified to express human genes or tissues—holds great promise for improving the predictive power of preclinical studies. These models may provide more accurate insights into human responses to drugs, thereby reducing the likelihood of failure in clinical trials.

Collaboration between academia, industry, and regulatory agencies will also be crucial in shaping the future of animal research. By fostering partnerships, stakeholders can work together to develop innovative research strategies, establish best practices, and promote the ethical use of animals in research.

Additionally, the ongoing development of alternative methods will likely continue to gain momentum. While it is unlikely that animal research will be entirely eliminated in the near future, a hybrid approach that combines traditional animal studies with advanced alternatives may emerge as a more ethical and efficient paradigm for drug discovery.

COMMITTEE FOR CONTROL AND SUPERVISION OF EXPERIMENTS ON ANIMALS (CCSEA): RULES & GUIDELINES

Prof. (Dr.) Manish Kumar
Professor, Dept. of Pharmacology,IGIMS, Patna

The Committee for Control and Supervision of Experiments on Animals (CCSEA), formerly known as the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), is a statutory body established under the Prevention of Cruelty to Animals (PCA) Act of 1960 by the Indian Parliament. Now operating under the Ministry of Fisheries, Department of Animal Husbandry and Dairying, Government of India, CCSEA is dedicated to ensuring that animals used in experiments are not subjected to unnecessary pain or suffering. To achieve this, the Committee has formulated the Breeding of and Experiments on Animals (Control & Supervision) Rules, 1998, with amendments in 2001 and 2006. The primary objective of CCSEA is to oversee the effective functioning of the Institutional Animal Ethics Committee (IAEC), ensuring a quality and consistent ethical review mechanism for all research proposals involving animals. The IAEC plays a crucial role in overseeing the ethical conduct of animal experiments, ensuring compliance with all regulatory requirements, and maintaining high standards of animal welfare.

The Institutional Animal Ethics Committee (IAEC) aims to ensure ethical practices in animal experimentation. Key objectives include:

1. Avoiding animal experiments whenever possible.
2. Preferring smaller lab animals over larger ones if similar results can be achieved.
3. Limiting the use of any animal for experimentation to a maximum of three years, unless adequately justified.
4. Prohibiting experiments solely for acquiring manual skills.
5. Ensuring experiments are conducted with care and humanity.
6. Requiring experiments to be performed or supervised by qualified professionals.
7. Using sufficient anesthetic agents to prevent pain.
8. Allowing animals to die under anesthesia to prevent suffering upon recovery.
9. Providing proper care for animals before, during, and after experiments.
10. Maintaining suitable records of all experiments performed on animals.

The Institutional Animal Ethics Committee (IAEC) is dedicated to achieving its objectives by ensuring ethical and methodical handling of animals during and after experiments to minimize suffering. Key functions include:

1. Approving and reviewing all research protocols involving small animals before the study begins.
2. Forwarding recommended proposals for large animal experimentation to CCSEA for approval.

3. Monitoring research throughout and after the study.
4. Obtaining periodic reports on research development and conducting visits to animal house facilities and laboratories.
5. Ensuring compliance with all regulatory requirements, applicable rules, guidelines, and laws.

Institutional Animal Ethics Committee (IAEC) Composition and Guidelines

The **Institutional Animal Ethics Committee (IAEC)** is a crucial component of the Committee for Control and Supervision of Experiments on Animals (CCSEA). The IAEC ensures ethical and humane treatment of animals used in research. Below are the key aspects of IAEC composition, authority, requirements, quorum, and nominee guidelines:

Composition: The IAEC must include a minimum of eight members:

- **Five members from the establishment**:
    1. One biological scientist
    2. Two scientists from different biological disciplines
    3. One veterinarian involved in animal care
    4. One scientist in charge of the animal facility
- **Nominees selected by CCSEA**:
    1. Main Nominee
    2. Link Nominee (substitutes the Main Nominee if unavailable)
    3. Scientist from outside the institute
    4. Socially Aware Nominee

Chairperson and Member Secretary should be nominated from the five internal members. If the administrative head of the establishment, who is from a non-scientific background, is proposed as Chairperson, six members may be proposed. The presence of a veterinarian is mandatory for assessing animal care and handling.

Authority & Requirements

1. **Registration**: IAEC is registered by CCSEA for five years.
2. **Revision and Renewal**: Requires CCSEA approval during the five-year period. Reconstitution is mandatory after five years, with at least half of the internal members replaced.
3. **Member Replacement**: Allowed in cases of death, long-term unavailability, resignation, or non-compliance with guidelines.
4. **Confidentiality**: Members must maintain confidentiality and sign a confidentiality form.
5. **Conflict of Interest**: Must be declared by members.
6. **Standard Operating Procedures (SOPs)**: IAEC must formulate and follow SOPs.
7. **Foreign Nationals**: Not allowed as IAEC members.

Quorum Requirements

- Minimum of six members required for quorum.
- Decisions on project proposals must be made during IAEC meetings.
- Establishments must inform CCSEA about continuous nominee absences.
- Minimum of two IAEC meetings per year for fewer than 40 research projects; four meetings for more than 40 projects annually.

Nominees

- **Invitation and Notice**: Must be sent to all nominees by registered post at least 15 days before the meeting.
- **Main Nominee**: Mandatory presence in each IAEC meeting.
- **Link Nominee**: Can attend if the Main Nominee is unavailable and has informed the Chairperson in writing.
- **Socially Aware Nominee**: Must attend at least one meeting per calendar year.

Institutional Animal Ethics Committee (IAEC) Conduct of Business

IAEC conducts its business under the leadership of the Chairperson, who is responsible for organizing at least two meetings annually with the assistance of the Member Secretary. In the Chairperson's absence or in cases of conflict of interest, an ad-hoc Chairperson is elected from the present members to conduct the meeting. The Member Secretary's duties include organizing meetings, maintaining records, and communicating with all concerned parties with the Chairperson's approval. The Member Secretary also prepares the minutes of the meetings, obtains approval from the Chairperson and members, and communicates these to researchers. A copy of the minutes must be sent to the Member Secretary, CCSEA, within 15 days of the meeting for it to be considered valid. The goal of CCSEA Guidelines is to promote the humane care of animals used in biomedical and behavioural research, providing basic provisions for animal care in teaching or research.

Concept of 5Rs in Rational use of animals in experiments

The CCSEA guidelines aim to promote humane animal care in research, emphasizing the **5Rs concept**. It guides the ethical use of animals in research and experimentation, originally described by W.M.S. Russell and R.L. Burch in 1959 with the 3Rs, and later expanded by CPCSEA in 2004. The principles are:

1. **Replacement**: Using alternative methods to avoid or replace the use of animals in research, preferring non-animal methods to achieve the same scientific aims.
2. **Reduction**: Employing methods that obtain comparable levels of information from fewer animals or more information from the same number of animals.
3. **Refinement**: Implementing techniques that minimize potential pain, suffering, or distress, and enhance animal welfare.

4. **Rehabilitation**: Providing aftercare for large animals used in experiments to alleviate pain, distress, or suffering, and ensuring a life different from laboratory conditions until natural death.
5. **Reuse**: Reusing animals in the same or different protocols after the completion of an experiment, with proper certification from a veterinarian, typically after a washout period of test drugs.

Facility and Care Standards:

1. **Bedding:** Must be absorbent, non-toxic, and replaced twice weekly.
2. **Sanitation:** Regular cleaning with appropriate detergents and disinfectants.
3. **Personal Hygiene:** High standards maintained with PPE for staff.
4. **Restraint:** Devices to minimize stress during examinations.
5. **Transport:** AC vehicles, suitable containers, and adequate feed and water.
6. **Physical Facilities:** Durable, moisture-proof, fire-resistant materials; wide corridors; accessible utilities.
7. **Animal Room Doors:** Non-rust, vermin, and dust-proof with observation windows.
8. **Exterior Windows:** Generally not recommended, except for light and ventilation during power failures.
9. **Floors, Walls, and Ceilings:** Smooth, moisture-proof, and resistant to chemicals.
10. **Storage Areas:** Separate areas for feed, bedding, and unused materials.
11. **Environment:** Controlled temperature, humidity, ventilation, light intensity, and noise levels.

Veterinary care: It is mandated, and animals must be procured from CCSEA-registered breeders. Quarantine procedures ensure the health of newly received animals, with specific periods for different species. Adequate nutrition and clean water are essential for animal welfare.

Record Keeping and Disposal:

- Detailed records of animal house plans, staff, health, SOPs, breeding, experiments, deaths, and clinical records.
- Euthanasia and proper disposal of animals as per SOPs.

Anaesthesia: Appropriate anaesthesia for painful procedures, ensuring animals do not perceive pain and remain under veterinary care until recovery.

Euthanasia (Painless Death):

- Approved methods should be used to minimize anxiety, pain, or distress.
- Conducted quickly in a separate, contaminant-free location.
- Should align with the study's purpose and minimize emotional impact on the operator.

HANDS ON TRAINING TO FILL THE FORM B (IAEC)

Prof. (Dr.) Manish Kumar
Professor, Dept. of Pharmacology,IGIMS, Patna

Dr. Manish Kumar, Additional Professor; Dr. Pankaj Prabhakar, Scientist I; Dr. Manoj Kumar, Veterinary Officer, Dept. of Pharmacology, IGIMS, Patna

The Committee for Control and Supervision of Experiments on Animals (CCSEA) is a statutory Committee of Department of Animal Husbandry and Dairying (DAHD), Ministry of Fisheries, Animal Husbandry and Dairying (MoFAH&D), Govt. of India. It was constituted under the Prevention of Cruelty to Animals (PCA) Act, 1960. Accordingly to CCSEA, each animal house establishment shall have CCSEA approved "Institutional animal ethical committee (IAEC)" for approving research protocol involving animal experiments, and supervising overall animal ethics in the establishment. Accordingly, CCSEA provided Forms A, B, C, and D which are to be duly documented and maintained at animal facility. It also noted that there total of 15 formats available in CCSEA website for easy documentation and monitoring. Among these formats, Form B (under section of 8a) is meant for "Research protocol submission by investigators to IAEC as an application for IAEC approval for conduct of experiments in animal, fish, etc. This Form B consist of three sections which includes, application (Section I and II), investigator declaration and certificate of ethics approval. Section I is about Institute/animal house establishment information whilst Section II describes about "Protocol form for research proposals to be submitted to the Institutional Animal Ethics Committee/ CPCSEA, for new experiments or extensions of on-going experiments using animals".There is a necessity for all investigators to avail the adequate knowledge before filling of Form B. It is always advised to visit CCSEA website from time to time for new update on animal ethics regulation, implementation regulation and procedure.

ARRIVE GUIDELINES

Dr. Ramesh Kumar Nirala, Associate Prof., Dept. of Veterinary Pharmacology & Toxicology, Bihar Veterinary College, Patna

The Recommended Set

Abstract	11	Provide an accurate summary of the research objectives animal species, strain and sex, key methods, principal findings, and study conclusions
Background	12	a. Include sufficient scientific background to understand the rationale and context for the study, and explain the experimental approach b. Explain how the animal species and model used address the scientific objectives and where appropriate, the relevance to human biology.
Objectives	13	Clearly describe the research question, research objectives and, where appropriate, specific hypotheses being tested.
Ethical statement	14	Provide the name of the ethical review committee or equivalent that has approved the use of animals in this study, and any relevant licence or protocol numbers (if applicable). If ethical approval was not sought or granted provide a justification.
Housing and husbandry	15	Provide details of housing and husbandry conditions, including any environmental enrichment
Animal care and monitoring	16	a. Describe any interventions or steps taken in the experimental protocols to reduce pain, suffering and distress b. Report any expected or unexpected adverse events C. Describe the humane endpoints established for the study. the signs that were monitored, and the frequency of monitoring. If the study did not have humane endpoints, state this
Interpretation/scientific implications	17	a. Interpret the results, taking into account the study objectives and hypotheses current theory, and other relevant studies in the literature. b. Comment on the study limitations including potential sources of bias, limitations of the animal model, and imprecision associated with the results
Generalisability/translation	18	Comment on whether, and how, the findings of this study are likely to generalise to other species or experimental conditions, including any relevance to human biology (where appropriate).
Protocol registration	19	Provide a statement indicating whether a protocol (including the research question, key design features and analysis plan) was prepared before the study. and if and where this protocol was registered
Data access	20	Provide a statement describing if and where study data are available
Declaration of interests	21	a. Declare any potential conflicts of interest, including financial and nonfinancial If none exist, this should be stated b. List all funding sources (including grant identifier) and the role of the funder(s) in the design,

RODENT BIOLOGY AND GENDER IDENTIFICATION OF LABORATORY ANIMALS, IDENTIFICATION OF SPECIES AND STRAIN

Dr. Manoj Kumar, Veterinary Officer, Dept. of Pharmacology, IGIMS, Patna
Dr. Bazla Nazir, PG Student, Dept. of Pharmacology, IGIMS, Patna

To study a human disease, it is not possible to perform the initial works in human for ethical reason, we have to develop and use animal model. There are various laboratory animal models available that includes mice, rat, guinea pig, rabbit, zebra fish etc.

1. Rat (Rattusnorvegicus):

The laboratory rat (Rattus norvegicus) is a mammal of the order Rodentia. The laboratory rat was the first animal in which the primary reason for domestication was for use in scientific endeavours. Rats are one of the most common (second to mice) laboratory animal used for research. Rats have several unique biological characteristics. The acute hearing of rats makes them sensitive to ultrasounds and high-pitched sounds. Rats have poor vision; they are unable to detect colour and are blind to long-wave (red) light. The rat's tail is the principal organ for heat exchange. Rats are basically docile, curious animals and usually develop closer bonds with humans than mice. Rats respond positively to quiet, gentle handling. Work quietly among the animals, and try to avoid performing procedures in the animal housing room. Wistar albino rat is white in colour and head is conical. Sprague dwaley rat is also albino strain which was developed from wistar rat and has wider head and longer tail length from wistar rat. It is more docile in nature so commonly used in pharmacology after wistar rat. Long-Evans rats are used for behavioural activity and obesity study. It is also known as hooded rat. Body colour is black and white. Rats have shorter tai length then body length as compared to mice.

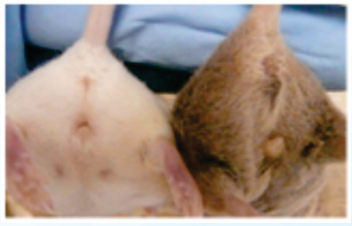
Wistar albino rat

Long evans rat (hooded rat)

Sprague dwaley rat

- **Sexing of rat:**

Male and female rat can be differentiated by observing the distance from the anus and genital papilla (called the **anogenital distance**) which is greater in males. This difference is also present in neonatal mice. In addition, one can usually determine gender by looking for the presence of testicles..



Sexing of Rat
(Left has lesser anogenital distance is female and right side rat has greater anogental distance is Male)

Identification

Cage cards are utilized to identify the strain of rat, sex, number, principal investigator, and IAEC protocol #. Cage cards should not be removed from the cage to avoid misidentification of the animals. Temporary identification of individual rats can be accomplished by pen marks on the tail, hair clipping or dyeing the fur. Pen marks will only last a few days whereas hair clipping may last up to 14 days. Ear punch identification and ear tags can be utilized but may be 5

obliterated by fighting between individuals. Microchips and tattoos have also been used for identification

Uses of Rat:

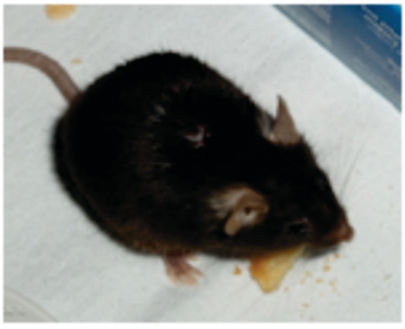
- ❖ Study of analgesics and anticonvulsants, toxicity screening of various drugs.
- ❖ Hepatotoxicity and mast cell study.
- ❖ Bioassay of various hormones like insulin, oxytocin, vasopressin etc.

Note : Method Sexing in Rat, Mice and Hamster are similar based on ano-genital distance.

1. Mice (*Musmusculus*)

The laboratory mouse (*Musmusculus*) is a mammal of the order Rodentia. Mice are the most common laboratory animal used for Biomedical research because of its small size, easy to handle, short life span, rapid reproduction rates, and low cost. Apart from these things it is the first species whose whole genome sequencing was done and it is almost 95 percent similar to human beings that will help to develop different disease model with the help of genetic engineering tools. Among mice, Swiss albino mice are the most commonly used strain. Other notable biological characteristics are their very acute hearing, well developed sense of smell, poor vision, small size and short generation interval. The laboratory mouse can be easily handled with appropriate training. Animals that grow up together or those grouped at weaning usually coexist peacefully. However, some strains of mice (i.e. BALB/CJ, SJL/J, HRS/J) may begin to fight even if grouped at weaning. Wounds on the tail or along the back are a common sign of aggression between cage mates. Mice, like most species, have a circadian rhythm. Investigators should be aware that this may affect biological data, and it is best to standardize the time of day that samples/measurements are taken to avoid this effect. The standard light/dark cycle in animal rooms. The small size and relatively large surface area/body weight ratio makes mice susceptible to changes in environmental conditions. The core body temperature is easily affected by small changes in ambient temperature, which may modify the physiologic responses of the animal. The acute hearing of mice makes them highly sensitive to ultrasounds and high-pitched noises inducing a stress response that has been empirically related to cannibalism of pups by their dams. The well-developed sense of smell is used to detect pheromones used in social interactions. Mice have poor vision and are unable to detect colour. Red light is often used to observe animals during the dark cycle.

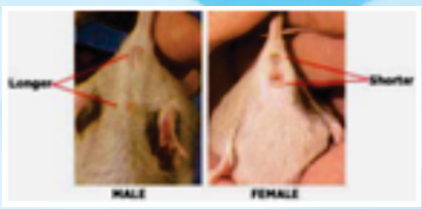
Swiss albino mouse



CD75BL/6 Mouse

Nude Mouse (Hairless)

Sexing of mice:



Sexing of mice

Male and female mice can be differentiated by observing the distance from the anus and genital papilla (called the anogenital distance) which is greater in males. This difference is also present in neonatal mice. In addition, one can usually determine gender by looking for the presence of testicles. However, one must realize that rodents have the ability to retract their testicles into the abdominal cavity (thus the apparent absence of testicles does not necessarily mean the mouse is a female).

Uses:

Investing physiology of mammals. They have similar reproductive and nervous systems to humans and suffer from same diseases as cancer, diabetes and anxiety.

- Toxicological study
- Teratogenicity bioassay of hormones
- Cancer and genetic study
- Drug action on CNS
- Screening of chemotherapeutic agents.

2. Guinea Pig (CaviaPorcellus)

Guinea pig

Guinea pig is very docile laboratory animal. It has well developed vocal cord which help in sound production to attract animal attendant attention when it need feed and water. It doesn't have tail. It has biological similarities to humans which make them suitable for research. Vitamin C was discovered through research on g. pig because it lacks gluconolactone oxidase enzyme which is essential for vitamin synthesis. Hence extra dietary supplementation is essential for G. Pig. Its blood components and isolated organ preparations such as lungs and intestines are extensively used in research to develop new medicines. Terminal portion of ileum used for screening spasmodic and anti-spasmodic agents. The allergic reaction and anaphylactic shock have been studies extensively in G.pig.

Sexing

when gentle pressure is applied over external genetalia, penis extrudes out in case of young male and Y shape genital fold of genitalia in case of young female. In case of adult male scrotal sac are seen and in case of adult female one pair of teats are seen.

Uses :

- Evaluation of local anaesthetics
- Vaccines for TB, Diphtheria etc.
- Nutritional research like vitamin C, Thiamine, potassium etc.

3. Rabbit (*Oryctolaguscuniculus*)

Rabbit was used for rabies vaccine development by Loius Pasteur earlier days. Most commonly used rabbit breedis New Zealand white(NZW). New Zealand white is an albino breed white in colour while Russian grey giant is greyish to brown in colour and chinchilla is cross of black and white.

Sexing of Rabbit:

when gentle pressure is applied over external genetalia, cylindrical shape penis extrudes out in case of young male and vulval lips are seen in case of young female. In case of adult male scrotal sac are seen and in case of adult female 5-6 pair of teats are seen.

Uses:

- Rabbit is used to develop various animal disease model like cancer, glaucoma, eye and ear infection, skin infection and emphysema. It can be used for pyrogen testing, bioassay of anti diabetic drugs, drugs used for glaucoma.

Reference:

1. Animalresearch.info [Internet]. Available from: https://www.animalresearch.info/en/
2. Aboutanimaltesting.co.uk [Internet]. Available from: https://www.aboutanimaltesting.co.uk/
3. Mice and rats[Internet]. Available from: https://informatics.jax.org/

IDENTIFICATION OF ANIMAL BEHAVIOR AND COMMON, SIGNS OF ILLNESS

Dr. Manoj Kumar, Veterinary Officer, Dept. of Pharmacology, IGIMS, Patna
Dr. Bazla Nazir, PG Student, Dept. of Pharmacology, IGIMS, Patna

Good science and good animal care go hand in hand. A sick or distressed animal does not produce the reliable results that a healthy and unstressed animal produces. For this careful observation of laboratory animal behaviour in their home cage is essential to get an idea about his health condition and welfare of animals. Activity like nest building, interaction with cage mates and general appreance are the indicator of general health and well-being.

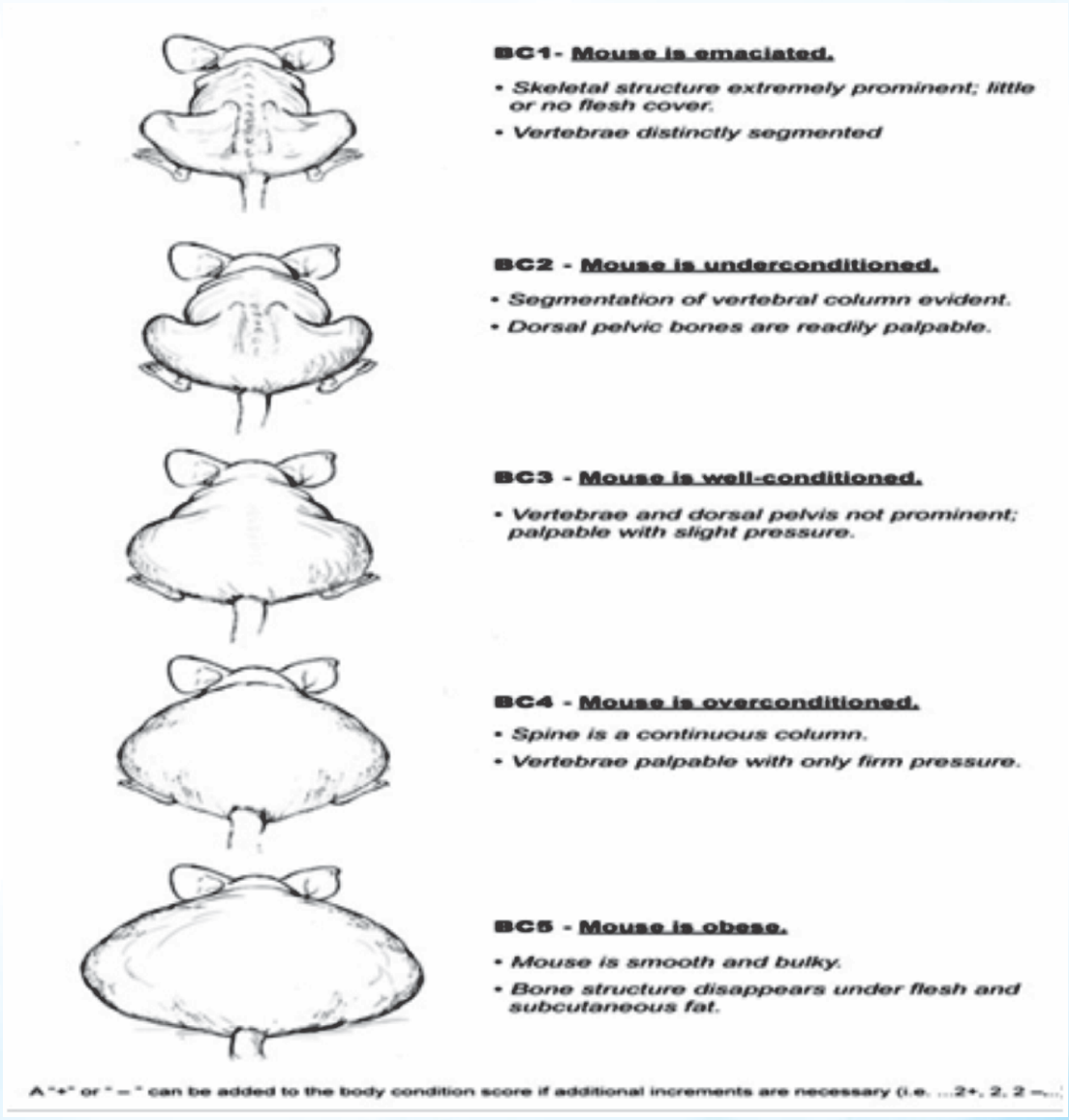


Figure 1: Body Condition Scoring (BCS) is a quick, easy and reliable method for assessing mouse health. It utilizes a scoring system of 1 to 5 with 3 being the optimal condition, 1 being emaciated and 5 being obese.

Animal can be assed based on body condition scored(BCS) as shown in figure 1. Body condition in mice by passing a finger over the sacroiliac bones (the spine and hip bones), and assign a score from 1 to 5. A score of 1 indicates emaciation, and a score of 5, obesity. In an optimally conditioned mouse/rat, scored as 3, the bones are palpable but not prominent. Figure 1, illustrates the appearance and the feel on palpation of the different levels of body condition. Body condition provides a more sensitive measure of welfare than body weight in the mouse because many common health conditions such as tumors can cause increases in body weight while breaking down body fat and muscle.

Rat and mice have common behaviour of foraging so in normal condition healthy animals groom each other hair looks like shiny and bright. If there is any stain or dirty hair coat that indicates animal is sick. In diseased condition animal will be isolated from colony, dirty hair coat, dehydrated, emaciated, lordosis and kyphosis posture etc.

- **Maintenance behaviour-** body care, foraging, feeding, drinking, nesting, sleeping, barbering (Figure.2).
- **Non social behaviour-** Digging, gnawing to prevent teeth overgrowing, climbing, territorial sent marking etc.
- **Social interaction-** huddling, aggressive, defensive etc.

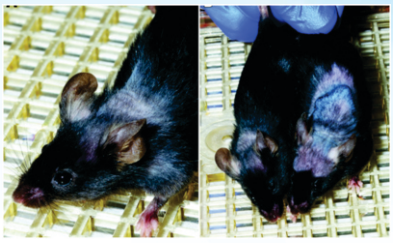


Figure 2. left figure showing less Barbering on the head and neck in mice while right figure showing two mice, one with minor and one with extensively barbered fur. Note that the skin is healthy in these cases.

Rats and Mice are nocturnal and thus are active primarily during the night at which time they feed. During the daytime, rats and mice tend to rest and sleep. Handling animals during the night can be more difficult due to this increase activity. The diurnal rhythm can be changed by a 12-hour shift in the light cycle. It takes approximately two weeks for rats and mice to adjust to this shift. Rats/Mice tend to get along well with other rats/Mice. However, please realize that introducing two rats/mice of the same sex to each other after weaning age can result in fighting and, potentially, serious injuries as shown in figure 3. Similarly, rats/Mice of the same sex that have been housed together may fight if separated and later re-introduced. Male and female rats/mice tend to accept each other. If one is to introduce post weaning rats/mice that are strangers, one should take the following precautions: Introduce the rats/mice together into a clean cage so that neither rat has established the cage as their home territory. Supervise the rats/mice closely over the next hour or so to see how they do. Be prepared with another cage to separate the rats/mice if needed.

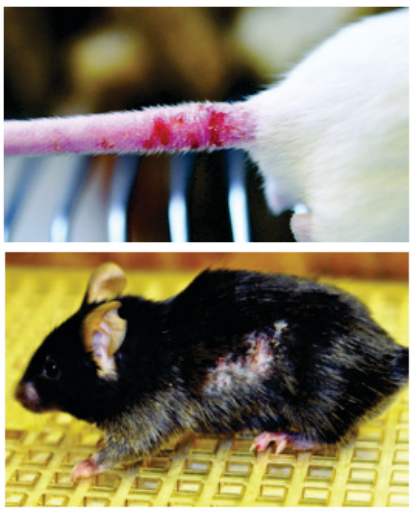


Figure 3: figure showing tail bite injury and injury/wound on body surface due to fight with each other.

While in case of severe pain or distress animal will shows certain facial expression, and body animal condition which can be identified by investigator or attendingveterinarian as shown in figure 4 and Table 1(bellow).

Eyes squinted, skin around nose contracted Ears pulled back

Figure 4: showing facial expressions in mice indicating pain and or distress

Table. 1 Assessing pain and distress in rodent

Pain and distress assessment	Examples
1. No indication of pain and distress	Normal; well groomed; alert; active; good condition; asleep or calm; normal appetite; BCS = 3,4, or 5
2. Mild or anticipated pain and distress	Not well groomed; awkward gait; slightly hunched; looks at wound or pulls away when area touched; mildly agitated; BCS = 2
3. Moderate pain and distress	Rough hair coat; dirty incision; squinted eyes; moves slowly; walks hunched and/or slowly; depressed or moderately agitated; slight dehydration; pruritic; restless; uncomfortable; not eating or drinking; BCS = 2–.
4. Severe pain and distress	Very rough hair coat; eyes sunken (severe dehydration); slow to move or nonresponsive when coaxed; hunched; large abdominal mass; dyspnoea; self-mutilating; violent reaction to stimuli or when approached; BCS = 1

Common Clinical conditions:

1. Rapid or progressive weight loss
2. Debilitating diarrhoea
3. Dehydration/reduced skin turgor
4. Oedema
5. Sizable abdominal enlargement or ascites
6. Progressive dermatitis
7. Rough hair coat
8. Hunched posture
9. Lethargy or persistent recumbency
10. Coughing, laboured breathing, nasal discharge
11. Jaundice, cyanosis, and/or pallor/anaemia
12. Neurological signs 13. Bleeding from any orifice
14. Self-induced trauma

15. Any condition interfering with daily activities (e.g., eating or drinking, ambulation, or elimination)

16. Excessive or prolonged hyperthermia or hypothermia

17. For aquatic species, additional signs can include scoliosis, emaciation, significant skin lesions, and/or exposure of muscle or other tissues

18. Lack of responsiveness to manual stimulation

Reference:

1. Balcombe JP. Laboratory environments and rodents' behavioural needs: a review. Laboratory Animals. 2006 Jul;40(3):217–35.
2. Burkholder T, Foltz C, Karlsson E, Linton CG, Smith JM. Health Evaluation of Experimental Laboratory Mice. Current Protocols in Mouse Biology [Internet]. 2012 Jun;2(2):145–65. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3399545/

ANIMAL HANDLING AND TECHNIQUES OF DRUG ADMINISTRATION & BLOOD COLLECTION

Dr. Pankaj Prabhakar, Scientist I, Dept. of Pharmacology, IGIMS, Patna
Dr. Amrendra Kumar Arya, Senior Resident, Dept. of Pharmacology, IGIMS, Patna
Dr. Ruhi Bhimseria, PG Student, Dept. of Pharmacology, IGIMS, Patna

Animal handling

Animal handling is a term that describes how persons work with, respond to, and interact with animals within their surroundings.The use of appropriate and skilled handling is essential to ensure that animals readily accept or actively seek human contact and procedures are carried out efficiently. Further, the correct handling techniques for laboratory animals minimize stress and risk of injury for both the animal and its human handler. Moreover, good animal handling reduces the risk of developing a zoonotic infection too (Ellenberger. 1993). Mishandling of animal is likely to develop anxiety and show exaggerated stress responses when approached to them. It has been observed that handle small animal has a major impact on whether or not familiarization with handling leads to a substantial reduction in aversion towards the handler, the stress experienced during handling, and the anxiety this induces (Hurst and West. 2010).

Animal should be approached in a relaxed and confident manner while handling the animals. They should be handled regularly to reduce stress among them.

Alexander Woods (1817-1884) developed hypodermic needle and proved that the effects of drugs can be achieved even if they are administered by parenteral route(Royal College of Physicians of Edinburgh; https://www.rcpe.ac.uk/heritage/college-history/alexander-wood). Administration of substances/drugs to small animals in the laboratory is a critical component of experimental design. Administered substances may comprise: infectious agents,numeroustherapeutics like vaccinations, antimicrobials, pharmacologic agents etc. Because substances/drugs may be administered frequently to the same animal or to multiple animals during the study. Therefore, the dosing methodology is an important concern when planning an experiment. Precise considerations for delivery of substances to animals are severalwhich include factors such as absorption, distribution, metabolism and excretion of therapeutic or chemical agents; route, volume, and frequency of administration.A key factor to determinethe route of administration is whether the agent is being administered for a local or systemic or parenteral effect (Turner et al. 2011).

Different route of administration of substances in small animals are: oral route, parental route [intravenous (IV), intramuscular(IM), intraperitoneal (IP) and subcutaneous (SC)] and other routes (topical, rectal, intrauterine) etc.

Oral administration

- ✓ Oral route is convenient and relatively safe
- ✓ Both solid and liquid dosage form can be given through this route
- ✓ Metal Gavage is inserted into the left side of the animal's mouth and directed along the hard palate of the mouth to the back of the throat

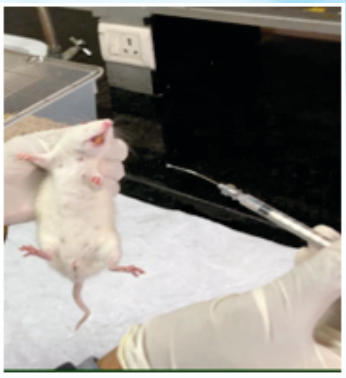


Fig. 1: Rats hold with one hand and gavage in left another hand

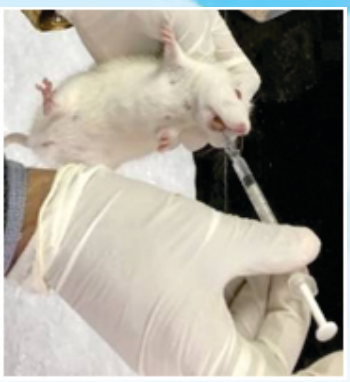


Fig. 2: Gavage is inserted into the side of the animal's mouth

Precautions: Common complications associated with oral administration are damage to esophagus and administration of drug into trachea. Careful and gentle passage of the gavage can reduce these issues. Optimum volume for oral administration should be 5 ml/kg (Turner et al. 2011).

Intraperitoneal administration (IP)

- ✓ Administration of substances/drugs into the peritoneal cavity is a common method in laboratory rodents but rarely used in larger mammals and humans.
- ✓ By this route sterile substance/drug solutions are injected into the peritoneal cavity in the gap between the wall of peritoneum and visceral organs.
- ✓ IP administration includes holding of the rodent in asupine position with its head tilted lower than the posteriorpart of the body and insertion of the tuberculin needle (1 ml, 26.5 G) in the lowerquadrant of the abdomen (Shoyaib et al. 2019).
- ✓ The plunger of syringe is pulled back to check the appropriate position of the needle. Injection should be given in the lower left quadrant of the abdomen. Note:
- ✓ By intraperitoneal route maximum up to10 ml/kg solution can be injected to rats (Turner et al. 2011).

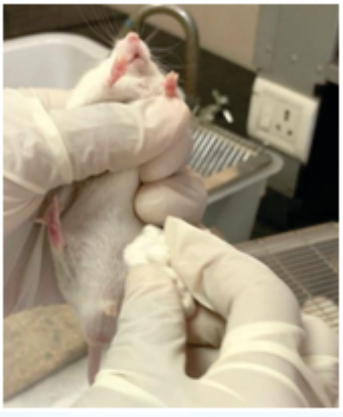


Fig. 3. A. Cleaning of lower left quadrant of the abdomen; **B.** Intraperitoneal route of administration

Subcutaneous administration

- ✓ The drugs which are highly active and are expected to get slowly absorbed are administered by this route.
- ✓ A small volume of substance/drug solution not more than5 ml/kg is administered by this route (Turner et al. 2011).
- ✓ For injecting the drug below skin, a fold is created in the skin by pressing it between the thumb and forefinger of one hand. The needle is inserted at the base of this fold at 20–30° angle. Preferable site for injection are the skin fold between two ears or dorsal sides near spinal cord (Turner et al. 2011; Shimizu. 2004).

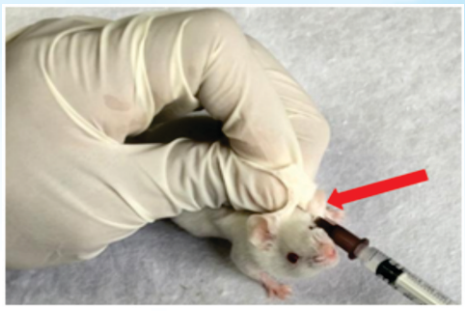


Fig. 4: Subcutaneous administration of substance/drug

Blood collection from the small laboratory animals

- ✓ The method of blood collection should be described in the study protocol approved by the Institute animal ethics committee.
- ✓ It should be least painful and stressful.
- ✓ Blood sample may be collected under anesthesia.
- ✓ It has been recommended that all nonterminal blood collection without replacement of fluids is limited up to 10% of total circulating blood volume [or 1% of body weight] in healthy, normal, adult animals on a single occasion and blood collection may be repeated after 3 to 4 weeks (Parasuraman et al. 2010).

Blood collection from Retro orbital sinus

- ✓ Requirements include animal, anesthetic agent, cotton, capillary tube and blood sample collection tubes.
- ✓ A fine capillary is inserted into retro orbital sinus by rotating it.
- ✓ Required amount of blood is collected and the capillary tube is gently removed from the retro-orbital sinus.
- ✓ After that wiped with sterile cotton & is pressed against the eye (Parasuraman et al. 2010).

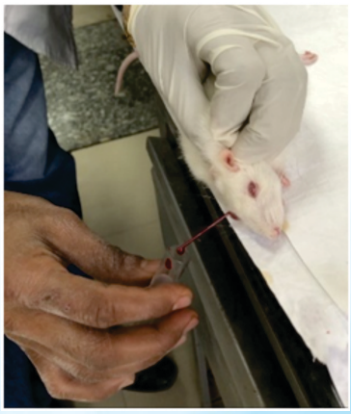


Fig. 5: Blood collection from retro orbital sinus

Blood collection from tail vein of rat

- ✓ This procedure can be carried out in the anesthetized rats/mice.
- ✓ The tail is dipped in warm water (about 40°C).
- ✓ It may be easier to have a 1 ml tuberculin syringe (26.5 G).The needle is inserted, in the distal/lateral portion of the tail vein (Parasuraman et al. 2010; Brown 2006).

Fig. 6: Blood collection from tail vein of rat

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SCREENING METHODS OF ANTI-INFLAMMATORY DRUGS

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Inflammation is one of the common events in the majority of acute as well as chronic debilitating diseases and represent a chief cause of morbidity in today's era of modern lifestyle. Current days anti-inflammatory drug discovery is based on preliminary in vitro observations followed by several in vivo methods, which together can mimic a broad spectrum of acute, sub-acute and chronic inflammatory events such as an acute, transient phase, characterized by local vasodilatation and increased capillary permeability. A subacute phase, characterized by infiltration of leukocytes and phagocytic cellsand a chronic proliferative phase, in which tissue degeneration and fibrosis occur. According to these phases, pharmacological methods have been developed. The prominent in vivo models of inflammation are carrageenan induced edema, pleural exudation & cotton pellet implantation. The methods of early pleural exudation and carrageenan induced edema assess the efficacy of compounds against transudative & exudative phase of inflammatory reaction respectively. On the other hand, cotton pallet implantation is used to study the efficacy of drugs against proliferative phase of inflammation. Various in vivo models of inflammation have been used summarized in table.

SL. No.	Model of inflammation	Animal used	Site of Application	Phase of inflammation assessed
1.	UV-B induced erythema	Guinea Pig	Depilated skin	Acute phase
2.	Carrageenan induced paw edema	Rats, Mice	Sub planter region	Acute phase
3.	Histamine-induced paw edema	Rats	Sub planter region	Acute phase
4.	Xylene induced ear edema	Mice	Ear	Acute phase
5.	Arachidonic acid induced ear edema	Mice	Topical application on ear	Acute phase
6.	Croton oil induced ear edema	Mice	Topical application on ear	Acute phase
7.	Oxazolone induced ear edema	Mice	Topical application on ear	Acute phase
8.	Papaya latex induced arthritis	Rats	Sub planter region	Acute phase
9.	Pleural exudation	Rats	Pleural space	Acute phase
10.	Carrageenan Air Pouch	Rats, Mice	Dorsal Surface	Acute phase
11.	Carrageenan-induced granuloma pouch	Rats	Dorsal Surface	Sub-acute phase
12.	Formalin induced paw edema	Rats	Sub planter region	Sub-acute phase
13.	Cotton pellet induced granuloma	Rats, Mice	Subcutaneous through the skin incision, groin region flanks	Chronic phase
14.	Glass rod granuloma	Rats, Mice	Back, Groin	Chronic phase
15.	Cotton wool granuloma	Rats	Subcutaneous through the skin incision, groin region flanks	Chronic phase

Carrageenan induced paw edema Model

This method is based on the principle of release of various inflammatory mediatory by carrageenan which leads to oedema formation in the rat paw and is considered as a biphasic event. The initial phase is attribute to the release of histamine and serotonin. The second phase of edema is due to release of prostaglandins, protease, and lysosome. The edema or swelling is measured by an instrument known as **Plethysmometer**. The instrument measures the volume of the rat paw in the presence and absence of irritant and after the treatment of anti-inflammatory drug. Carrageenan in this model can be replaced by other irritant such as formalin, mustard oil, snake venom, dextran and polyvinylpyrollidone etc.

Methods:

1. After overnight starved, Male or Female Sprague-Dawley or Wistar Albino rats are divided in three groups:
   - **A. Control group**: Given normal saline orally 1hr before procedure
   - **B. Standard group**: Given standard drug i.e. indomethacin (8mg/kg) orally 1hr. before the procedure.
   - **C. Test group:** Given drugs to be tested by recommended route 1hr before the procedure

2. A mark is made at ankle joint of each rat.
3. 0.05 ml of 1% carrageenan is injected s.c. into the plantar region of one paw (right or left) of each rat of each group.
4. 0.05 ml of normal saline is injected into another paw of each rat as control
5. Paw volume upto ankle joint of ach paw upto ankle joint of each rat is measured with help of Plethysmometer at 0hr, 3hr, 6hr. and 24hr. after carrageenan injection and recorded in following observation table.

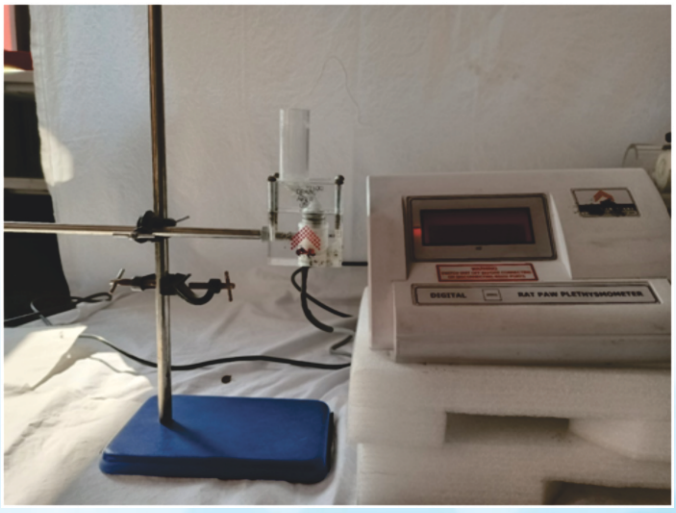

Figure 1: Digital Rat Paw Plethysmometer

Observation Table

Sl. No.	Paw Edema (0hr)		Paw edema (3hr)		Paw Edema (6hr)		Paw Edema (24hr)	
	Right Paw	Left Paw	Right Paw	Right Paw	Right Paw	Left Paw	Right Paw	Left Paw
1.								
2.								
3.								
4.								
5.								
6.								

% Reduction in edema = [(Vc-Vt)/Vc]×100

Whereas, –

Vc=Mean edema in control group

Vt=Mean edema in treated group

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DEMONSTRATION OF SCREENING OF EFFECT OF DRUGS ON WOUND HEALING

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Summary

Management of a Wound has always been challenging for the medical community, whether the nature is acute, accidental, or chronic. Empathy has always been to find a suitable strategy to heal the wound in an appropriate way. Even though a lot of research has been done, the hunt for a proper healing strategy is still ongoing. Suitable preclinical wound healing models have always supported wound healing research. The studies included in vitro using various skin-based cell lines and in vivo studies using rodent models. These models help to understand the factors that influence wound healing as well as to evaluate the efficacy of treatments applied to wounds. The demonstration will include preclinical/animal models including incision, excision, burn and impaired wounds. A brief discussion will be on the methods to evaluate the healing progression, using wound healing rate, wound analysis by image, biophysical and biochemical assessment, and histopathological and immunological aspects. [1]. Impaired wound conditions include delayed wound healing models using steroids like dexamethasone, diabetes and others.

A. ***In vitro* study:**

Cell lines: Fibroblast, Keratinocyte; **Studies**: Cell viability assay for safety of test materials, Cell proliferation assay using scratch wound to assess epithelialization rate.[1], ther mechanistic studies

B. ***In vivo* studies:**

B.1. Excision model: It is the most commonly used wound healing model in resemblance to the acute conditions of clinical wounds. In this condition skin edges are not sutured. All skin layers, including epidermis, dermis and subcutaneous fats, are removed. In this model, we assess haemorrhage, inflammation, granulation tissue formation, reepithelialization, angiogenesis and remodelling. Various species of animals used are mice, rats, rabbits and pigs etc. The number of wounds created varies based on the size of the animals, type of excision and diameter of the biopsy punch, such as up to two wounds per mouse, up to four wounds per rat and four or more per pig and rabbit.**Procedure**: In this model, rats or animals are anaesthetized with ketamine+ xylazine. The back of the rat is shaved to remove fur. The impression is made, and skin is excised of 500 mm2 on the dorsal thoracic central region and 5 cm away from the ears. After hemostasis animals were kept in cages. Drug is applied as per schedule. The tracing of images use to be taken on tracing paper until the scab is removed. [2]

Parameters: Percentage of Wound contraction; Period of Epithelialization: Measured in the form of the days for falling of scab; Histopathology of the skin

B.2. Incision Model: This model can be used for investigating the properties of

sutures and the potency of test items for wound healing properties by measuring tensile strength. Briefly, animals were anesthetize with ketamine and xylazine. The incision is made in two para-vertebral straight lines of 6 cm on either side of the entire thickness of the skin with 1 cm lateral to the vertebral column. The sutures are placed equidistant by 1 cm each. Animals are treated. Sutures are removed on the 7^{th} wounding day. Wound breaking strength is estimated on the 10^{th} day post wounding using constant water flow technique using pully and bottle.

B.3. Dead Space model: A dead space is created by implanting a polypropylene tube in the lumbar region on the dorsal side. The polypropylene tube dimension generally is 2.5 cm×0.5 cm. On the 10^{th} day, animals are sacrificed, and the breaking strength of granular tissues is estimated using the constant water flow technique. Using a microplate reader,the hydroxyproline level is estimated. The wet granulation tissue is used for lysyl oxidase estimation and other estimations for the promotion of granulation.

B.4. Burn Wound Model: Thermal damage to the skin is created by using molten waxes/hot water/ hot metal plates in a limited area. The remaining procedures are the same as the excision wound model.[3]

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DEMONSTRATION OF SCREENING OF ANTIEPILEPTIC ACTIVITY

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Epilepsy is a common disorder. It is characterised by abnormal or excessive in neuronal activity in brain, with or without characteristic body movements. The usual approach of screening model of anti-convulsant drug testing in animal is to observe the effect of prior drug administration on seizures.

It is produced mainly by various ways like Electrical stimulation of brain, Systemic administration of the convulsant drugs, Kindled rat seizure model, Genetic rat model seizure etc.

(A) Electrical stimulation of brain: Electrical shock given through the electrodes applied on the ear pinna results into a burst of excitatory neurotransmitters from the brain. This activates the brain activity during grand-mal epilepsy.

(B) PTZ induced Epilepsy: Pentylenetetrazol is a CNS Stimulant. It produces jerky type of clonic convulsion in rats and mice similar to petit mal type of convulsion in man due to antagonizing inhibitory GABAergic transmission.

(C) Isoniazid induced convulsion: Seizures occurs due to insufficient inhibitory action of GABA or extreme excitation of glutamate. Isoniazid is regarded as GABA synthesis inhibitor and causes convulsion.

(D) Picrotoxin induced epilepsy: Picrotoxin is $GABA_A$ - antagonist modifying the function of chloride ion channel of $GABA_A$ receptor complex. It induces seizures in animals and epileptiform activity in brain slice preparations.

(E) Strychnine induced convulsion: Glycine is inhibitory neurotransmitter. Strychnine act as selective and competitive antagonist to block inhibitory effect of glycine to all glycine receptors. Administration of strychnine causes convulsion due to interference with post synaptic inhibition mediated by glycine.

(F) Kindled seizure model: Repetitive administration of sub convulsive electrical stimulation of certain areas of brain causes focal seizure. On continued stimulation, electrical activity spreads and generalized convulsions occur.

(G) Genetic model: Genetically Epilepsy Prone Rats (GEPR), DBA/2J mice - audiogenic seizures.

Prior treatment of animals with the drugs, reduces the exited activity of brain. These models can be used for the discovery and characterization of spectrum of anticonvulsant activity of new anti-epileptic drugs.

DEMONSTRATION OF SCREENING OF ANALGESIC ACTIVITY

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Introduction

Pain is an unpleasant sensory and emotional experience associated with actual and potential tissue damage. There are various types of pain are seen in human, e.g. Somatic pain, visceral pain, referred pain, neuropathic pain, cancer pain, etc. Drugs in clinical use as analgesics belong to two main groups – narcotic or morphine group and analgesic-antipyretic (NSAIDs) group. There are various in-vivo methods used to demonstrate analgesic effect of drugs. The last decade has witnessed an explosion of information on transmitters, receptor and channels involved in the transmission and modulation of noxious stimuli generated in the peripheral tissue. Numerous animal models have been evolved to screen novel drugs for their analgesic activity.

Various in vitro methods used to assay potential analgesic drugs include 3H-Naloxone binding assay, 3H-Dihydromorphine binding to opiate receptor in rat brain, 3 Bremazocine binding to k opiate receptors in guinea pig cerebellum, inhibition of enkephalinase, and receptor binding and bioassays of nociceptin.

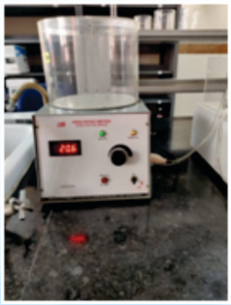
Common animal models of acute pain according to types of stimuli –

Thermal	Mechanical	Chemical	Electrical
Tail-flick test	Haffner's tail clip method	Formalin test (Intradermal inj.)	Tail stimulation
Hot-plate test	Randall and Selitto test	Writhing test (Intraperitoneal inj.)	Tooth pulp test
		Chemical stimulation of visceral organs	

Acute pain models- in vivo methods:

Painful reaction production by applying noxious (unpleasant) stimuli such as

- Thermal (radiant heat as source of pain)
  - Eddy's hot plate method



- Tail-flick method- using analgesiometer
- Tail flick using immersion of tail in warm water
- Cold tail flick test
- Cold ethanol tail flick test

▫ Chemical (irritants such as acetic acid and byadykinin)

▫ Physical (tail compression) electrical

Thermal method

- The method deals on the principle of the thermal conduction or radiation heat. This principle is used in the animal experiment for the evaluation of the centrally acting analgesics and hence this method found to be the differentiating between the centrally acting opiates and non-opiates analgesics.

1. Eddy's hot plate method

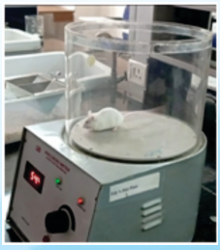
Introduction: The method was first described by Woolfe Eddy & Mac Donald (1944) as modified by Eddy and Leimbach in 1953 has been used frequently. The paws of mice and rats are very sensitive to heat at temperatures which are not damaging the skin. The responses are jumping, withdrawal of the paws and licking of the paws. The method deals on the principle of the thermal radiation heat. This principle is used in the animal experiment for the evaluation of the centrally acting analgesics.

Method: The instrument involved is known as "hot plate analgesiometer". Instrument consists of an electrically heated surface (made up of iron, aluminum or copper) whose temperature is maintained by the thermostat 'Knob' at 55 ˚ to 56 ˚ C.

· White Albino mice are divided in three groups:

a. Control Group: Given normal saline 1 ½ hr before
b. Standard group: Pentazocine (0.5 mg/kg)
c. Test Group: Given drug to be tested by recommended route 1 ½ hr before procedure.

Step 1: Mice weighing 18-40 gm placed on the hot plate and the time until either paw licking or jumping occurs is recorded by a stop watch. All other signs of discomfort such as kicking are disregarded. The latency is recorded before and after 15, 30, 60 and 90 minutes following oral or subcutaneous administration of the standard or the test compound. The prolongation of the latency times comparing the values before and after the experimental groups can be used for statistical comparison using the t- test.



Step 2: The response is considered positive when the reaction time after injection is longer than 30 seconds at least once, or when three or more recordings exceed the normal reaction time by a factor of at least three. The cut off time for rat is 20-30 second and for mice it is 15-20 second.

The method has the drawback that sedative and muscle relaxants

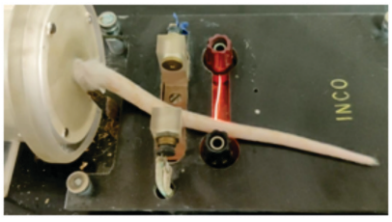
(Woolfe and McDonald 1944) or psychomimetics (Knoll 1967) cause false positives, while mixed opiate agonist – antagonists provide unreliable results. Hot plate test is useful only for detecting narcotic analgesics. However, in a procedure devised by Jacob Boscvski (1961) if the temperature of the plate is increased to 65 ˚C, the analgesic action of non-narcotic drugs like aspirin can be evaluated.

2. Tail-flick method- using analgesiometer

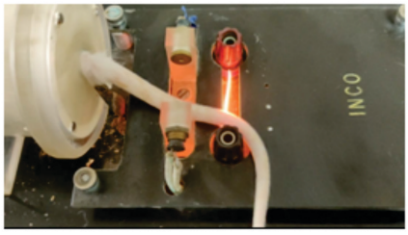
Tail flick examination was used to calculate analgesic activity by the method defined by D'amour and Smith 1941, with minor alterations in the procedure.

Method: The tail flick method was utilized to study the antinociceptive activity in mice.

Step 1: Mice or Rat is placed into restrainer and leaving the tail exposed outside the restrainer. Clean the tail with the help of cotton soaked in water or ethanol and keeps it for drying and also to settle down the rat/mice in restrainer.



Step 2: When animal settled, and then keep restrainer on the "tail flick analgesiometer". 1/3rd tail proximally left due to the thick and keratinized skin and then keep tail on the place made for tail above the hot wire (measure the height of tail from wire) of the analgesiometer.



Step 3: The time of tail flick is measured and recorded. The cut off time is set up 15-20 second in case of mice whereas in the case of rat, cut off time is 20-30 second to avoid any further injury to the tail.

Observation Table

Group 1: Control (Normal saline)
Group 2: Std. (Pentazosine 0.5 mg/kg)
Group 3: Test (Eth SR 400)

A. Eddy's hot plate method

Group	Dose /kg	Reaction time before drug administration	Response Time [paw licking or jumping] (after __ hrs of administration)			
			15 min	30 min	60 min	90 min
Control						
Standard						
Test						

A. Tail-flick method- using analgesiometer

Group	Dose /kg	Reaction time before drug administration	Response Time [tail flick] (after __ hrs of administration)			
			15 min	30 min	60 min	90 min
Control						
Standard						
Test						

References:

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DEMONSTRATION OF SCREENING OF ANTI-ANXIETY ACTIVITY

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Anxiety is subjective human phenomenon. The heightened level arousal and subjective feeling of fear and features of all major categories of anxiety disorders. It is regarded as particular form of behavioral inhibition that occurs in response to environmental events. Stimuli associated with punishment, non-punishment and novelty elicit inhibition of ongoing behavior increase attention to environment and increase alertness have face validity as description face human anxiety. However, the validity of animal model of anxiety is depending upon when the model can discriminate between effective and ineffective treatments. The validity increases if the model not only discriminates active from inactive compounds but also the potency correlated with clinical potency. These models are used to test new anxiolytic and helps in understanding neural mechanism.

Anxiety model can bases on

1) Exploratory 2) Social 3)Defensive 4) Novelty evoked 5) Conditioned (active/passive avoidance 6) Andedonicbehavior and 7) Condition fear related behaviors.

Commonly used models are listed below.

Exteroceptive stimuli model (stimuli originating outside body)	Interoceptive stimuli model (stimuli originating inside within body)
<u>Response independent presentation of stimuli</u> • Open field test • Staircase exploration • Y-maze • Elevated plus maze • Head dipping(hole-board) test • Food consummate exploration • Black and white test box • Mirrored chamber <u>Inescapable aversive events</u> • Conditioned emotional response • Potentiated startle • Conditioned acceleration <u>Response-contingent presentation of stimuli</u> • Behavior suppression by aversive stimuli Geler-seifter conflict test • Vogel conflict test • Quintero punished bar pressing • Behavior acceleration by aversive stimuli • social interaction behaviour	<u>Electrical stimulation of brain</u> <u>Pharmacological manipulation</u> • Fg-7142-induced anxiety • Caffine-induced anxiety • Yohimbine--induced anxiety • Flumazeline-induced anxiety • Pentylenetetrazole-induced anxiety • Amphitamine-induced anxiety • Piperoxane-induced anxiety • Cocaine-induced anxiety <u>Aggression/ Anxiogenesis</u> • Foot shock-induced aggression • Drug shock-induced aggression • Instillation shock-induced aggression

Elevated plus maze (Pellow et al 1985)

The underlying principle in this paradigm is that the open arms are more anxiety-provoking and ratio of either time spent in open arm to closed arm reflects the relative safety of closed arms compared with relative fearfulness open arms.

The maze consists of two open arms (length 50cms X breath 10cms) and two closed arms of same size with 40cm high wall arrangement so that arms of same type are opposite to each other with central square of 10cms. The maze is elevated to height of 50cms above the floor.

Ideally At least 6 animals in each groups (control, standard and test) should be incorporate in study. Albino wister,150-180g body weight)is placed in centre square of maze facing one of enclosed arms. Number of entries into and time spent in open and closed arm will be noted. Number of rears in each arm and duration of immobility to be noted during the 5 minute observation. Fecal pellets are counted.

Precautions before experimentation

- Laboratory should be dim light and noise free
- Animal should be marked properly to avoid mixing
- Handle the animal with care to avoid stress and pain
- Place the rat at centre of maze, facing towards open field
- Expose rat to the experiment procedure 1-2 day prior to the experiment
- Video recorder is placed to record the experiment in calm and dim lighted room

Observations:

Treatments	% of open/total arm entries ratio	Time spent in sec		No. of rears in	
		Open arms	Closed arms	Open arms	Closed arms
Control 1% Gum acacia					
Alprazolam 0.08mg/kg					
NR-ANX-C 10mg/kg					

Bright and dark Arena (costall et al 1988:crawley 1981)

Rodents show natural aversion to bright area when compared to darker area. Exposure to bright light is believed to be a noxious environment stressor. Mice or Rats tends to explore a novel environment but they retreat from aversive sight of bright lit arena. Animals are paradigm two chambered system where they freely move between the bright and dark chamber. Reduction in number of entries, decrease time spent and decrease exploratory behavior in bright area are regarded as marker of anxiety in the paradigm. Classic anxiolytic (benzodiazipines)as well as newer anxiolytic-like compounds(e.g. seronerergic drugs or drugs acting on neuropeptide receptor)can be detected using this paradigm . It has advantage of being quick and easy to use, without requiring of prior training of animals.

The apparatus consist of an open top wooden box with two distinct chambers, a black chamber(20cmX30cmX35cm)painted black and illuminated with dimmed red light with a bright camber(30X30X35cms)painted white and brightly illuminated with 10W white light source. Tho chambers are connected through a small open doorway.

Animal((Albino wister,150-180g body weight)is placed at the center of bright lit arena. Number of

transitions between chambers and time spent in bright arena, number rears in dark and bright arena and duration of immobility to be noted during 5 minute observation.

Observations:

Treatments/kg	No. OF ENTRIES TO BRIGHT ARENA	TIME SPENT IN BRIGHT ARENA IN SECONDS	REARS IN BRIGHT ARENA	REARS IN DARK ARENA	TIME SPENT IN IMMOBILITY
Control1%GAC10ml					
Standered, Alprazolam(0.08 mg)					
Test ,NR-ANX-C(10mg)					

Reference:

1. Drug discovery and evaluation, pharmalogicalassays, H.Gerhard. wolfgangH.Vogel, publication Springer-verlag Berlin Heidelberg 1997 p234
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DEMONSTRATION OF SCREENING OF EFFECT OF DRUGS ON NEUROMUSCULAR COORDINATION

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Aim

To demonstrate the muscle relaxant property of diazepam in mouse / rat using rotarod apparatus.

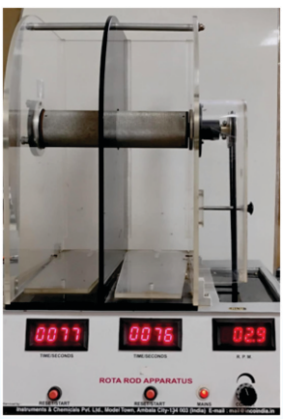
Background

Rota-rod test is used to measure fore limb and hind limb motor coordination of rodents. The device is a horizontal metal rod with a diameter of 3 cm that is covered in rubber and coupled to a motor that can be set to rotate at a speed of 2 or 6 revolutions per minute. The 75-centimeter rod can be split into four halves for testing six mice at once. To frighten, the animals from escaping the device, the six portions of the rod are separated by plastic discs, allowing it to be raised to a height of around 50 cm above the tabletop. The cut off time for the test is 2 min. The retention time (sec) for each mouse/rat is recorded.

Dimensions of Rota Rod

Mouse: The rod measures 75 cm in length, 30 mm in diameter, and is situated around 50 cm above the table top. It is separated into 6 portions by plastic discs.

Rat: A 75 cm long, 60 mm diameter rod that is 50 cm above the table top and split into 6 sections by plastic discs.



Rota-rod apparatus

Materials and Methods

Materials

Animal/species: Mice/Swiss albino

Sex/Body weight: Either sex/20-30 g

Syringe/needle: 1ml/preferably 24G on-wards

Drug: Diazepam (3-5 mg/kg, i.p.)

Diazepam

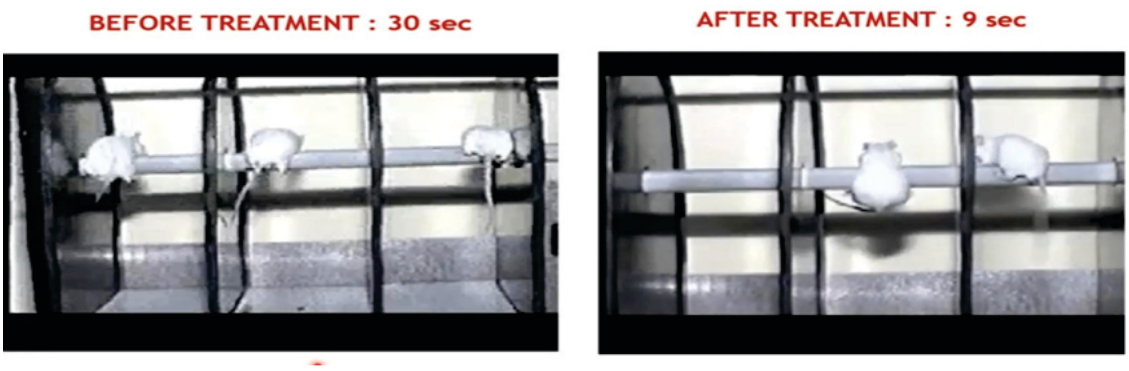
Precautions before Experimentation

Animals should be appropriately labeled to prevent mixing them into two groups; the laboratory should have low lighting and no noise.

Treat the animals with kindness, attempting to reduce their suffering and discomfort.

Use the protocol to precondition the mouse.

Methods



Step 1

Weigh the animals and mark properly to differentiate from one another

Divide animals into two groups (n = 6 in each group)

Step 2

Group 1: Control group (n = 6); mice are given saline at the equivalent dose of drug

Group 2: Treatment group (n = 6); mice are given diazepam at the dose of 3 mg/kg, ip

Step 3

Observe the animals for 2 min on the rota rod apparatus

Observe the animals, to fall down and note the time of falling down

Observations:

Sr. No.	Body weight (g)	Treatment	Fall off time (sec)		
			Before drug	After drug	% change in time
1	22	Diazepam	32	4	
2	20	Diazepam	31	8	
3	22	Diazepam	33	6	
4	23	Diazepam	29	4	
5	24	Diazepam	30	9	
6	20	Diazepam	33	7	
Mean					

Discussion:A vital connection between the brain processes that control the movement of a limb and the limb itself is achieved through the physiological process of motor coordination. The peripheral nervous system (PNS) is primarily responsible for processing this. It is in charge of both limb movement and the efferent's transmission to the central nervous system. Additionally, the CNS is essential for combining efferent impulses and afferent feedback. The rota-rod method, chimney test, grip strength, treadmill performance, and other experimental models are used to investigate the motor coordination of rats. Rats / mice are assessed for their ability to maintain muscular coordination, such as in the rota-rod test.

To demonstrate muscle coordination, the mouse / rat should stay on the rotating rod or climb backward throughout the chimney test. Benzodiazepam, chlordiazepoxide or dizepam, zolpidem, zopiclone, and other centrally acting skeletal muscle relaxants are among the medications that can be checked for using these assays.

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1. Cartmell SM, Gelgor L, Mitchell D. A revised rotarod procedure for measuring the effect of antinociceptive drugs on motor function in the rat. J Pharmacol Methods. 1991;26(2):149-59.
2. Dunham NW, Miya T S. A note on a simple approach for determining neurological deficits in rats and mouse. J Am Pharmacol Assoc. 1957;46:208.
3. Liu X, Lee TL, Wong PT. Cyclooxygenase-1 Inhibition Shortens the Duration of Diazepam- Induced Loss of Righting Reflex in Mouse. AnesthAnalg. 2006; 102:135-40.
4. Stanistaw J. Czuczwar, Kinga K. Borowicz, Zdzisław Kleinrok, Piotr Tutka, Tomasz Zar- nowski, Waldemar A. Turski. Influence of com- bined treatment with NMDA and non-NMDA receptor antagonists on electroconvulsions in mouse. Eur J Pharmacol. 1995;281:327-33.

HOW TO CALCULATE LD50

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LD50 or Median Lethal Dose is defined as the "dose of a given drug which produces mortality in 50% of total treated animal, preferably in the most sensitive species model."

Various methods exist for calculating the LD50. Each with distinct advantages and limitations. Among these, Karber's method and graphical approaches are prominent due to their straight-forward execution and reliability.

Calculationof LD50 value

- Graphical Method of Miller and Tainter (1944) (Probit-Analysis method)
- Arithmetical Method (Karber's Method)

Let us take an example of acute toxicity study to calculate LD50 value by the above two methods (Table-1).

Table-1: Example

Experimental Group	*Number of animals in each group(n)*	*Dose (mg/kg)*	*Number of animals died*
1	10	20	0
2	10	40	3
3	10	60	6
4	10	80	9
5	10	100	10

I. Karber's Method: It is a simplest and rapid method when number of animal is small. There is no need to plot the dose-response curve. The outlined steps for LD50 calculation are as follows:

i. The interval mean of the number dead i.e. mean mortality (b)) in each group of animals is used as well as the difference between the doses for the same interval i.e. dose difference (a) is derived.

ii. The product of the dose difference (a) and the mean mortality(b) is obtained.

iii. The sum of the product is divided by the number of animals in a group and the resulting quotient is subtracted from the least lethal dose (LD100) in order to obtain the LD50 value.

Formula for LD50 calculation: $LD50 = LD100 - (\sum axb)/n$

In this formula-

LD100 = Least lethal dose which produce 100% mortality

a = Dose difference between two consecutive doses
b = mean mortality from two consecutive doses
n= No. of animals in each group
Let us calculate LD50 value of taken example which is simplified in the Table- 2.

Table-2: Calculation of LD50 by Karber's method

Exp. Group	*No.of animals (n)*	*Dose (mg/kg)*	*Dose difference (a)*	*No. of animals died*	*Mean mortality (b)*	*Product (axb)*
1	10	20	-	0	-	-
2	10	40	20	3	1.5	30
3	10	60	20	6	4.5	90
4	10	80	20	9	7.5	150
5	10	100	20	10	9.5	190
Totaproduct (axb)						460

Now, putting the value in the formula,LD 50 = LD100 - (∑axb)/n;
LD50= 100-(460)/10 = 100-46 = 54 mg/kg approx.

I. **Graphical method:** The graphical method for LD50 determination is another widely utilized approach that enables researchers to visualize the relationship between dose and mortality. This method involves plotting the cumulative mortality against the logarithm of the dose administered to the animals, creating a dose-response curve.

The outlined steps for the graphical method are as follows:

I. The observed percentage mortality is converted into probit by referring to the Table-3 of Finney's table of transformation of % mortality in to probit*.
ii. The probit values thus obtained are plotted against log dose.
iii. Before plotting, the percentage mortality for zero and 100 are corrected (corrected percentage) by the formula;
For the zero % dead: 100 (0.25/n); for the 100 % dead: 100[(n-0.25)/n], where n is the number of animals in the group.
iv. The observed percentage mortality (corrected %) is converted into probit by referring to the Table-3.
v. The LD50 value and its standard error may be determined from the graph if the line is straight enough.
vi. The log dose corresponding to probit 5 (50%) is found.
vii. Antilog of value is LD50.

Table-3. Finney's table for transformation of percentage of mortality to probit values (Finney, 1952).

%	0	1	2	3	4	5	6	7	8	9
0	-	2.67	2.95	3.12	3.25	3.36	3.45	3.52'	3.59	3.66
10	3.72	3.77	3.82	3.87	3.92	3.96	4.01	4.05	4.08	4.12
20	4.16	4.19	4.23	4.26	4.29	4.33	4.36	4.39	4.42	4.45
30	4.48	4.50	4.53	4.56	4.59	4.61	4.64	4.67	4.69	4.72
40	4.75	4.77	4.80	4.82	4.85	4.87	4.90	4.92	4.95	4.97
50	5.00	5.03	5.05	5.08	5.10	5.13	5.15	5.18	5.20	5.23
60	5.25	5.28	5.31	5.33	5.36	5.39	5.41	5.44	5.47	5.50
70	5.52	5.55	5.58	5.61	5.64	5.67	5.71	5:74	5:77	5.81.
80	5.84	5.88	5.92	5.95	5.99.	6.04	6.08	6.13	6.18	6.23
90	6.28	6.34	6.41	6.48	6.55	6.64	6.75	6.88	7.05	7.33

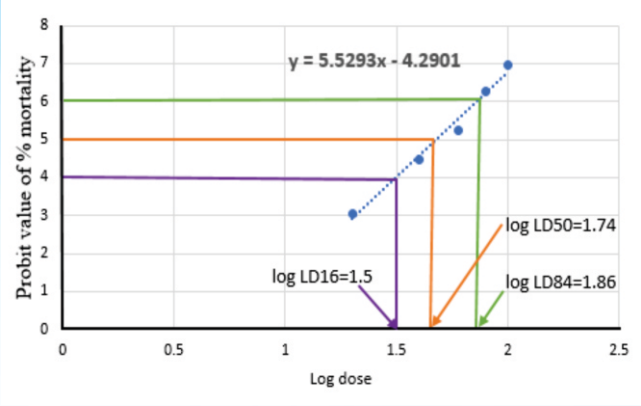
Table-4: Calculation of LD50 value by Probit -Analysis method

Exp. Group	*No. of animals (n)*	*Dose (mg/kg)*	*Log dose*	*No. of animals died*	*Dead %*	*Corrected %*	*Probit*
1	10	20	1.30	0	0	2.5	3.04
2	10	40	1.60	3	30	30	4.48
3	10	60	1.77	6	60	60	5.25
4	10	80	1.90	9	90	90	6.28
5	10	100	2	10	100	97.5	6.96

The probit values are plotted against log-doses and then the dose corresponding to probit 5, i.e. 50%, is find out. (Figure-1).

Figure-1: Plot of Log doses vs Probits

In the present case the log LD50 value is 1.74 and hence the antilog of 1.74 is the LD50 i.e. 54.95 mg/kg which is approximately same as the result obtained by Karber's method.



Calculation of Standard Error (SE) of LD50

The approximate standard error of LD50 value is obtained by the formula:

Approx. S.E. of LD50= (log LD84 – log LD16)/$\sqrt{2n}$ where n is number of animals in each group.

The probits of 84 and 16 from table-3 are 5.99 and 4.01 (Approx. 6 and 4), respectively. The log dose values for the probits 6 and 4 are obtained from the line on the graph in figure- 1, which in the present example are 1.86 and 1.5. Using these values in formula of SE of LD50 is 0.0783 and its antilog is 1.20. Therefore, LD50 of the taken example is 54.95 ± 1.20, with 95% confidence interval of 53.75 – 56.15.

Note:

1. Probit: A unit of measurement of statistical probability based on deviations from the mean of a normal distribution.
2. Probit analysis is a type of regression used to analyse binomial response variables. It is most precise but requires at least two groups of partial responses (i.e. mortality greater than 0% but less than 100%). Initially, Bliss (1939) developed the idea of probit through transforming the sigmoid dose-response curve to a straight- line dose-response curve which was further refined by Finney (1952)

References:

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BIOASSAY OF HISTAMINE BY THREE POINT ASSAY

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INTRODUCTIN: -Bioassay is defined as the estimation of the concentration or potency of a substance by measuring its biological activity on a suitable living organism (whole animal, organ or tissue).

Bioassays are mainly used to quantify biological substances for which established chemical or other assay procedures are NOT available. This experiment demonstrates graded dose response, interpolation method, bracketing assay and matching assay of histamine on the guinea-pig ileum.

Objectives: - To obtain a dose response curve of histamine on the guinea pig ileum

Animals and tissues commonly used in bioassay	Physiological salt solutions commonly used in bioassay
1) guinea pig ileum 2) Rectus abdominis muscle 3) Rat colon 4) Rat uterus 5) Rat vas deferens	1) Tyrode 2) Krebs 3) De jalon 4) Frog Ringer 5) Ringer Locke 6) Mc Ewen

Types of lever:- A) Simple lever
B) Frontal writing lever
C) Starling's heart lever
D) Brodie's lever

Types of common methods :-

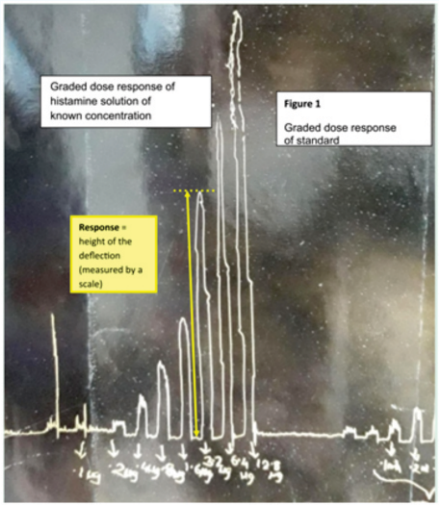
To determine the concentration of histamine in given solution using

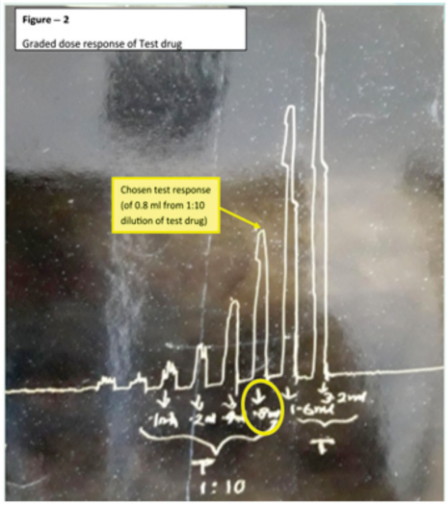
A) Interpolation method
B) Bracketing assay
C) Matching assay
D) Multiple point assay

Methods/Procedure for 3 point assay: -The guinea pig is sacrificed by a blow on the head and carotid bleeding. The abdomen is cut open and viscera inspected. In the right lowest quadrant lies a grayish sac like structure. The caecum is lifted to trace ileocaecal junction. Afewcentimeter of ileal portion is cut and removed and immediately placed in a petridishcontaining warm tyrode solution. The mesentry is trimmed gently and the contents of the ileum are cleaned by pushing Tyrode solution into the lumen of the ileum ut most care should be taken to avoid damage to the gut muscle. The ileum is then cut into segment 2-3 cm long and thread is tied to top and bottom ends taking care not to occlude lumen. The tissue is now mounted in organ

bath containing Tyrode solution with the lower thread the ileum is fixed to the bath and the upper end is tied to the recording lever. Temperature is maintained at 32-35 degree C and the solution is bubbled with air A tension of 0.5 Gm is applied and the tissue is allowed to equilibrate for 30 minute before addition ……

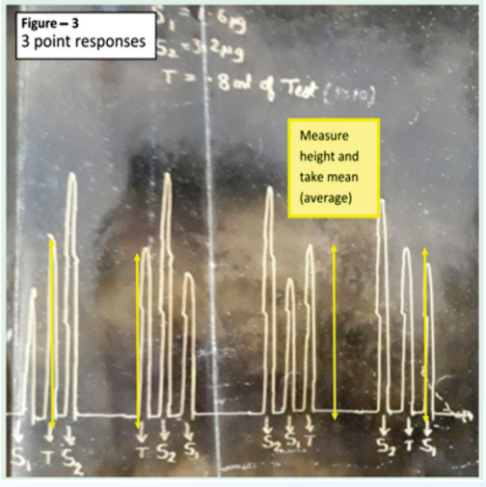
Graded strengths (dilution1:10,1:100, 1:1000)are used to elicit concentration dependent response due to Histamine contact time of 30 sec and 3 minute cycle are kept for proper recording of response





Then dose response to gradually increasing concentration of test drug (unknown) are recorded as below:

Then for 3 point assay, select suitable volume of test (T) insuch a way that the response due to the test lies betweenselected dose of S1 and S2. All these selected response shouldlie on the linear portion of the standard DRC. Record four setsof response due to S1 and S2 and T byadding them into theorgan bath in random fashion. The mean response ore noted asS1, S2 and T.



Latin square design:Two stardrds (S1 and S2)and one test concentrationare chosen These are administered repeatedlyaccording to Latin Squaredesign S1 T S2.

Formula for calculation

Potency ratio (M): X1/Y1 Antilog {(T-S1)/(S2-S1) x logX2/X1}

DETERMINATION OF PD2 OF HISTAMINE ON GUINEA PIG ILEUM

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Objectives

- To record the dose response curve for histamine on an isolated piece of guinea pig ileum
- To plot the log-molar concentration vs. percent response curves for histamine
- To determine the half maximal effective concentration (EC50) of histamine for guinea pig ileum
- To determine the PD2 value of histamine on guinea pig ileum

The study of pharmacodynamics is crucial for understanding the interaction between pharmacological agents and their biological targets. Histamine, a biogenic amine involved in various physiological functions, has garnered extensive attention due to its role in allergic responses, gastric acid secretion, and neurotransmission. Moreover, histamine acts on several receptor subtypes, primarily H1 and H2 receptors, leading to diverse physiological effects. Among the experimental models utilized in pharmacodynamic studies, the isolated Guinea pig ileum has been widely employed to investigate the contractile effects of histamine and to quantify the potency of pharmacological antagonists through the determination of PD2 values (negative logarithm of the molar concentration that produces a 50% maximal response.

Method

The determination of the PD2 value of histamine on the guinea pig ileum is conducted through in vitro experiments involving isolated tissue preparations. This methodological approach includes several critical steps, as outlined below.

1. Preparation of Guinea Pig Ileum:

Guinea pigs are euthanized humanely in accordance with ethical guidelines, and the ileum is excised. The tissue is then placed in a physiological saline solution, typically Tyrodesolution, maintained at 37°C to simulate physiological conditions. The ileal segments are subsequently cleaned and prepared for mounting in an organ bath setup.

2. Mounting in Organ Bath:

The ileum is mounted in an organ bath containing Tyrode solution, aerated to maintain physiological pH. One end of the ileal segment is tied to a force transducer

to measure contractions, while the other end is held stationary. The tissue is allowed to equilibrate for a period of approximately 30 minutes to ensure stable baseline activity.

3. Administration of Histamine:

Histamine dilutions are prepared in physiological saline, and the cumulative concentration-response curve is generated by applying increasing concentrations of histamine to the organ bath at specific intervals. Typically, concentrations ranging from 10^{-9} M to 10^{-4} M are tested to span the range of response.

4. Measurement of Response

- Start with the minimum possible dose (like 0.05 mL of 1 µg/mL).
- Each subsequent dose should be doubled (or should be increased in a geometric proportion, that means, the quotient of two subsequent doses should be constant).
- When the first measurable response (a response that can be measured for example, at least 5 mm height, or at least 10 mg force) is achieved, repeat the same dose and see whether you get a reproducible response. This proves the reproducibility of the response and proper acclimatization of the tissue to ex-vivo conditions.
- Record the responses of geometrically increasing doses till the maximum response is achieved (the subsequent doses give equal responses).
- Ideal doses are 1 µg/mL (0.05 to 0.8mL), 5 µg/mL (0.05 to 0.8mL), 10 µg/mL(0.8 mL), 20 µg/mL(0.8 mL)
- Once the maximum response is achieved, do not repeatedly expose the tissue to maximal dose or higher doses. Such repeated exposures may affect the responsiveness of the tissue.
- The capacity of the organ tubes is 10 mL.

The determination of the PD2 value requires the construction of a concentration-response curve based on data gathered during the experimentation. The calculated percentage of response is plotted against the logarithm of histamine concentrations to obtain a sigmoidal curve, which can be analyzed as follows:

1. Estimating the ED50:

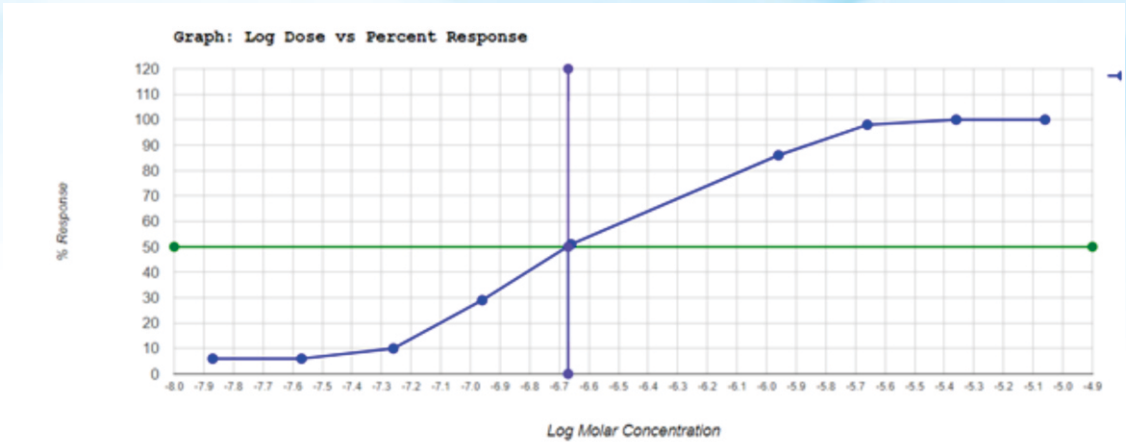
The effective dose that produces 50% of the maximal response (ED50) can be established from the sigmoidal curve. This point is fundamental as it signifies the concentration at which half of the maximum contractile effect is achieved.

2. Calculating PD2 Value:

The PD2 value is derived from the equation:

$PD2 = -LOG_{10}(ED50)$

This logarithmic transformation allows for a standardized quantification of drug potency, facilitating comparative analyses between various compounds and receptor systems (5).



Conclusion

The determination of the PD2 value of drugs on the contractile tissues provides insightful data into the pharmacological mechanisms underlyingreceptor interactions. Through carefully designed experimental protocols, including preparation of tissue, administration of agonists and antagonists, and appropriate statistical analyses, researchers are equipped to elucidate the potency and efficacy profiles of drugs and its modulators. Such methodologies not only enhance our understanding of histamine signaling pathways but also lay the groundwork for the development of novel therapeutic agents targeting histaminergic systems.

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DETERMINATION OF PA2 OF ATROPINE USING ISOLATED RAT ILEUM PREPARATION (BY SCHILD PLOT METHOD)

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Objectives

- To plot the DRCs of Acetylcholine on rat ileum in presence of incresing concentrations of Atropine
- To derive the Schild plot for Atropine
- To determine the PA2 of Atropine on rat ileum

Introduction

The pharmacological effects of atropine are primarily mediated through its antagonistic action on muscarinic acetylcholine receptors.The Schild plot method serves as a valuable tool in quantifying the affinity of competitive antagonists, such as atropine, for specific receptors by generating a relationship between drug concentration and the physiological response elicited by agonists in the presence of the antagonist.

Method

The experiment to determine the pA2 value of atropine was accomplished using an isolated rat ileum preparation, which is a classic model for studying muscarinic receptor-mediated responses. The following steps outline the process involved in the experimentation:

Step 1: DRC in absence of atropine

- Start with the minimum possible dose of acetylcholine (like 0.05 mL of 1 µg/mL).
- Each subsequent dose should be doubled (or should be increased in a geometric proportion, that means, the quotient of two subsequent doses should be constant).
- When the first measurable response (a response that can be measured example, at least 5 mm height OR at least 10 mg force) is achieved, repeat the same dose and see whether you get a reproducible response. This proves the reproducibility of the response and proper acclimatization of the tissue to ex-vivo conditions.
- Record the responses of geometrically increasing doses till the maximum response is achieved (the subsequent doses give equal responses).
- Ideal doses for acetylcholine are 1 µg/mL (0.05 to 0.8mL), 5 µg/mL (0.05 to 0.8mL), 10 µg/mL (0.8mL), 20 µg/mL (0.4mL), 40 µg/mL (0.2mL).

- Doses of Atropine (in presence of which the DRCs of Acetylcholine are to be plotted) .
- Once the maximum response is achieved, do not repeatedly expose the tissue to maximal dose or higher doses. Such repeated exposures may affect the responsiveness of the tissue.
- The capacity of the organ tubes is 10 mL.

Step 2: DRC of Acetylcholine in presence of Atropine

- To record the DRC of Atropine in presence of Atropine, we have to take first dose of Atropine. For this we can start with a dose of Atropine (example 0.1 mL of 0.5 µg/mL).
- This dose of atropine will be added to the organ tube and after 2-3 minutes of the dose of Acetylcholine will be added. The response to this dose of Acetylcholine will be recorded and then a wash will be given.
- Again, the same dose of atropine will be added and a response of next dose (increased in geometric proportion) of acetylcholine will be recorded.
- Thus, in presence of 0.1 mL of Atropine (0.05 µg/mL), complete DRC of Acetylcholine will be recorded.

Step 3: DRC of Acetylcholine in presence of next dose (increased in geometric proportion) of Atropine.

- Now record the DRC of acetylcholine in presence of higher concentration of Atropine (example 0.2 mL of 0.5 µg/mL).

Step 4: Record at least 3 DRCs of Acetylcholine in presence of increasing doses of Atropine.

- Preferably, 0.1 mL, 0.2 mL and 0.3 mL of 0.5 µg/mL of Atropine.

Step 5: Determine the ED50s for all the DRCs of Acetylcholine (the doses of Acetylcholine at which 50 % of the maximal response is achieved)

Calculation:

Analysis of Data: For each concentration of atropine, the corresponding acetylcholine concentration eliciting the same contraction level was noted. From this information, the dose-ratio (R) for each atropine concentration was calculated using the formula:

$$R = \frac{[A]}{[A']},$$

where ([A] is the concentration of acetylcholine required to produce a defined contraction in the presence of atropine, and ([A']) is the concentration that produces the same response in the absence of atropine.

Schild Plot Construction: A Schild plot was then constructed by plotting the logarithm of the dose ratios (log R) against the logarithm of the concentration of atropine used. The slope of the line was typically close to unity when the antagonist

acted competitively. The x-intercept of the regression line provides the negative pA2 value, which is indicative of the potency of the antagonist.

Apply linear regression to the transformed data of log R versus log [atropine]. The regression equation can be represented as follows:

$$\log R = m \cdot \log[\text{atropine}] + b,$$

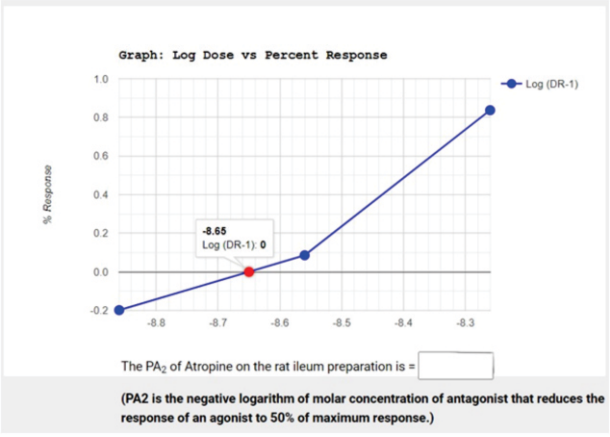
where m corresponds to the slope (ideally 1 for competitive antagonism) and b is the y-intercept.

The x-intercept, $-\log K_b$, where K_b is the equilibrium dissociation constant for the antagonist-receptor complex, will yield the pA2 value:

$$pA2 = -b.$$

Conclusion

The pA2 value derived from the Schild plot method offers critical insights into the competitive antagonism exhibited by atropine on muscarinic receptors in isolated rat ileum preparations. Understanding such metrics not only enhances our comprehension of atropine's pharmacological profile but also contributes to the broader understanding of receptor-ligand dynamics crucial for drug development and therapeutic applications. Future studies employing similar methodologies could further elucidate the intricacies of drug-receptor interactions across various biological systems.



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DEMONSTRATION OF EFFECTS OF DRUGS ON HR & BP OF DOG USING COMPUTER ASSISTED LEARNING (CAL)

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The exploration of pharmacological effects on cardiovascular system is pivotal for medicine and research. The experimentinvolves the utilization of Computer Assisted Learning (CAL) modules to simulate and understand the impacts of various drugs on heart rate (HR) and blood pressure (BP) in canines. Traditional methods of studying these effects often involve live animal testing, which can raise ethical concerns and pose logistical challenges. Computer Assisted Learning (CAL) presents an innovative approach, offering an interactive and ethical alternative.The CAL approach offers a safe, ethical, and interactive educational platform for students and researchers to explore drug responses without the need for live animal experimentation.

A series of simulations were developed to demonstrate how different classes of drugs, such as beta-blockers, vasodilators, and diuretics, affect the cardiovascular parameters of dogs. The modules enabled users to observe real-time changes in HR and BP with variable dosing schedules and drug combinations, thus providing insights into the complex pharmacodynamics and pharmacokinetics involved.The study utilized advanced CAL modules designed to simulate the physiological and pharmacological responses of dogs to various cardiovascular drugs. Key drug classes included Cholinergic, Anticholinergic, Adrenergic, Antiadrenergic and Vasodilators.

The findings from users of the modules showed increased understanding and retention of concepts related to cardiovascular pharmacology. Additionally, the simulation approach allowed for repetitive experimentation and exploration without the ethical concerns and variability associated with live animal testing.Each simulation module was developed to provide users with a dynamic interface where they could adjust drug dosages, observe real-time physiological changes, and interpret the effects through graphical data output. Scenarios included normal, hypertensive, and hypotensive states to illustrate drug impact under varying baseline conditions. This approach also allowed for repetitive trial-and-error interaction, facilitating deeper exploration into drug effects without the variability and stress associated with live models.

In conclusion, CAL serves as an effective pedagogical tool in pharmacology education, enhancing comprehension of drug effects on cardiovascular health while adhering to ethical standards in scientific research.

MYCALPHARM software is used for simulation for demonstrationof procedures/maneuvers and effects of drujgs on a liveanaesthetized dog for undergraduate students of medical,veterinary, dental,nursing, pharmacy, medical laboratorytechnology and physiotherapy. This software simulates the effectoffollowing procedures/maneuvers and drugs on the bloodpressure (BP) and Heart Rate (HR)and it aims to remember

andrecall the effects

a) Carotid occlusion
b) Electrical stimulation of cut end of vagus nerve
    i) Peripheral cut end of the vagus nerve
    ii) Central cut end of the vagus nerve
c) Agonists- like epinephrine (adrenaline), norepinephrine (noradrenaline), isoprenaline, ephedrine, acetylcholine, histamine
d) Antagonists- like phentolamine, propranolol, atropine, mepyramine, cimetuidine

Students can perform these procedures/ maneuvers as well asinject drugs and observe their effect (s) on this simulatedexperiment on live anaesthetized dog.

Experiment in Tutorial mode

Drugs are injected one by one and following parameters areobserved

a) Blood Pressure (mm of Hg)
b) Heart Rate (beats per minute)

Parameters a) and b) are assessed by observing the recording. The BP recordings are indicated in black and that of HR in red. Two scales for measuring BP and HR are provided one at each side of the chart. The one in which the measurements are marked in red indicates HR (beats/min) and the other with black marking indicates BP (mm of Hg). Moving the mouse pointer over the chart will display a crosshair that helps to measure the correct BP and HR.

Apart from drugs, a few procedures such as carotid occlusion, and electrical stimulation of cut ends of vagus can be performed to see the changes that these procedures would bring about on the BP and the HR.

1) The drug can be selected by clicking the mouse on the drug list. On selection, the selected drug and the recommended dose to be injected are displayed. The list includes carotid and vagus procedures also.
2) Always use the appropriate dose. The conventional dose range and the recommended dose for different drugs will be displayed. The dose can be altered by the user and the drug is injected by clicking the "Syringe" icon. Double clicking the drug name (on the drug list) will also inject the recommended dose of drug.
3) Br careful while selecting the dose. Low doses may not produce any effect. Whereas higher doses might kill the dog. Do not repeat the same blocker often. It might kill the dog.
4) The effect of blockers can be removed by pressing 'Remove Blocker' blocker. Names of the blockers in the action and their amount in the body will be displayed. User can remove any or all blockers. The level of blockers (concentration) will automatically decrease with time.

5) For 'Epinephrine' dose as low as 0.1mg/kg can be given to see the beta receptor action and for 'Ach' doses as high as 150-200mg/kg can be given to see the nicotinic action (after giving recommended dose of Atropine).

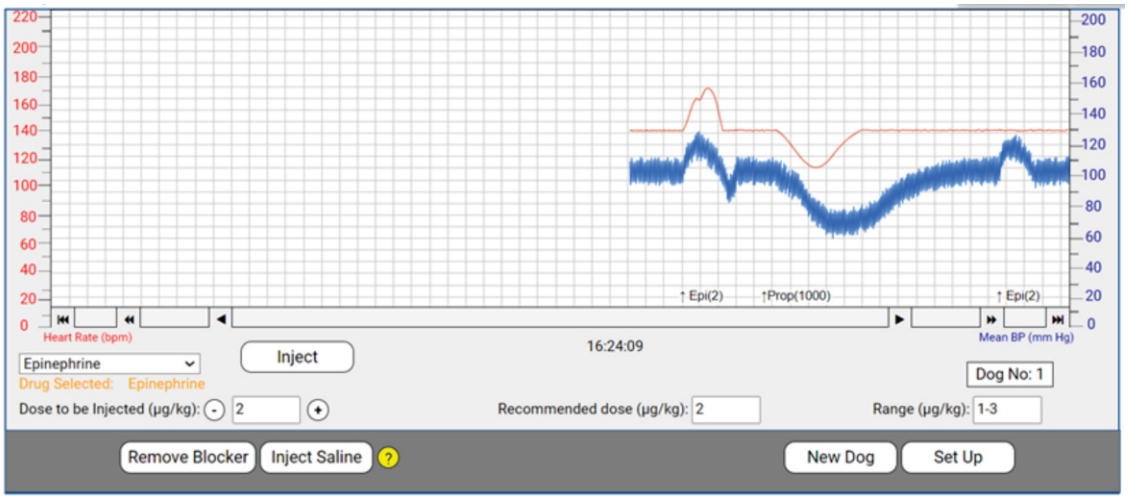
6) Start with carotid and vagal procedure and continue with agonists- This is called 'Normal Response Bracket or Normal Panel'. Then administer a blocker followed by the corresponding agonist to see the “agonist-antagonist” interaction.

Depending on the drug injected, a question will be displayed. The user is expected to find out the answer and write it in his/her record book.

Note: Two or more drug cannot be administered simultaneously; the drugs have to be given one after another only. The response to agonists can be tested while the antagonists are still present and acting on the body.

The recording can be saved, viewed or deleted using the options (button) under 'Session'.

Experiment in examination mode-Users have to identify the unknown procedure or drug based on their effect and interaction with other procedure or drugs.



DEMONSTRATION OF SOXHLET AND OTHER RELATED APPARATUSES

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Dr. Keshav Kumar, Senior Resident (DM Clinical Pharmacology Student), Dept. of Pharmacology, IGIMS, Patna-14

IMPORTANT TERMINOLOGY

***Medicinal plant*:** It refers to a plant comprising active ingredients or secondary metabolites that possess biological activity.

***Menstruum*:** It is a liquid or a suitable solvent chosen for an effective extraction process.

***Marc*:** It is an insoluble or inert drug material that is left behind at the end of the extraction process.

***Micelle*:** It is the mixture of both the extracted drug material and the solvent of extraction.

***Primary plant constituents*:** These are mainly nutritional components of plants such as common sugars, amino acid, proteins, and chlorophyll. These have little or no medicinal properties.

***Secondary plant constituents*:** These are also known as secondary metabolites such as alkaloids, terpenoids, saponins, phenolic compounds, flavonoids, and tannins. These are responsible for many biological or pharmacological activities.

METHODS USED IN EXTRACTION

Factors to be considered in choosing extraction method

(a) *Stability to heat*: Heat-stable plant material is extracted using Soxhlet extraction or microwave-assisted extraction, whereas plant materials that are not heat stable are extracted using maceration or percolation.

(b) *Nature of solvent*: If the solvent of extraction is water, maceration is a suitable method but for volatile solvent percolation and Soxhlet extraction are more appropriate.

© *Cost of the drug*: Cheap drugs are extracted using maceration, whereas costly drugs are preferably extracted using percolation.

(d) *Duration of extraction*: Maceration is suitable for plant material requiring long exposure to the menstruum, whereas techniques such as microwave- or ultrasound-assisted extraction are used for a shorter duration.

(e) *Final volume required*: Large volume products such as tinctures are prepared by maceration, whereas concentrated products are produced by percolation or Soxhlet extraction.

(f) *Intended use*: Extracts intended for consumption by human are usually prepared by maceration, whereas products intended for experimental testing are prepared using other methods in addition to maceration.

Commonly used methods in the extraction

1. *Maceration*
2. *Digestion*
3. *Decoction*
4. *Percolation*
5. *Soxhlet extraction*
6. *Microwave-assisted extraction*
7. *Ultrasound-assisted extraction*

SOLVENTS FOR EXTRACTION

The choice of solvent depends on the type of plant, part of plant to be extracted, nature of the bioactive compounds, and the availability of solvent. In general, polar solvents such as water, methanol, and ethanol are used in extraction of polar compound, whereas nonpolar solvents such as hexane and dichloromethane are used in extraction of nonpolar compounds. Solvent used in extraction is classified according to their polarity, from *n*-hexane which is the least polar to water the most polar.

Factors to be considered in selecting solvents of extraction

1. ***Selectivity***: This is ability of a chosen solvent to extract the active constituent and leave the inert material.
2. ***Safety***: Ideal solvent of extraction should be nontoxic and non-flammable.
3. ***Cost***: It should be as cheap as possible.
4. ***Reactivity***: Suitable solvent of extraction should not react with the extract.
5. ***Recovery***: The solvent of extraction should be quickly recovered and separated from the extract.
6. ***Viscosity***: The solvent should be of low viscosity to allow ease of penetration.
7. ***Boiling temperature***: Solvent boiling temperature should be as low as possible to prevent degradation by heat.

Sl. No.	Solvent	Polarity
1	n- Hexane	0.009
2	Petroleum ether	0.117
3	Diethyl ether	0.117
4	Ethyl acetate	0.228
5	Chloroform	0.259
6	Dichloromethane	0.309
7	Acetone	0.355
8	n- Butanol	0.586
9	Ethanol	0.654
10	Methanol	0.762
11	Water	1.000

Properties of solvents

1. ***Water***: It is the most polar solvent and is used in the extraction of a wide range of polar compounds.
   - ***Advantages.*** Dissolves a wide range of substances; is cheap, nontoxic, non-flammable.
   - ***Disadvantages.*** It promotes bacterial and mould growth; it may cause hydrolysis, and a large amount of heat (because of high boiling point) is required to concentrate the extract.
2. ***Alcohol***: It is also polar in nature, miscible with water, and could extract polar secondary metabolites.
   - ***Advantages.*** It is self-preservative at a concentration above 20%. It is nontoxic at low concentration, and as small amount of heat is required for concentrating the extract.
   - ***Disadvantages.*** It does not dissolve fats, gums, and wax; it is flammable and volatile.
3. ***Chloroform.*** It is a nonpolar solvent and is useful in the extraction of compounds such as terpenoids, flavonoids, fats, and oils.
   - ***Advantages.*** It is colourless, has a sweet smell, and is soluble in alcohols.
   - ***Disadvantages.*** It has sedative and carcinogenic property.
4. ***Ether***: It is a nonpolar solvent and is useful in the extraction of compounds such as alkaloids, terpenoids, coumarins, and fatty acids.
   - ***Advantages.*** It is miscible with water, has low boiling point, and is tasteless in nature. It is also a very stable compound and does not react with acids, bases, and metals.
   - ***Disadvantages.*** It is highly volatile and flammable in nature.
5. ***Ionic liquid (green solvent)***: This is highly polar and extremely heat stable.

   It can remain in a liquid state even at 3,000 ˚C (suitable where high temperature is required). It has extreme miscibility with water and other solvent and is very suitable in the extraction of polar compounds.
   - ***Advantages.*** It attracts and transmit microwave, and hence it is suitable for microwave-assisted extraction. It is non-inflammable.
   - ***Disadvantage.*** It is not ideal for preparation of tinctures.

SOXHLET EXTRACTION APPARATUS

The apparatus is called Soxhlet extractor (diagram 1) made up of glass and consists of a round bottom flask, extraction chamber, and condenser at the top. Soxhlet extraction process is otherwise known as continuous hot extraction. A dried,

grinded, and finely powdered plant material is placed inside porous bag (thimble) made up of a clean cloth or strong filter paper and tightly closed. The extraction solvent is poured into the bottom flask, followed by the thimble into the extraction chamber. The solvent is then heated (using heating mantle) from the bottom flask, evaporates, and passes through the side (evaporation) tube to condenser where it condenses and flow down to the extraction chamber and extracts the drug by coming in contact. Consequently, when the level of solvent in the extraction chamber reaches the top of the siphon, the solvent, and the extracted plant material flow back to the flask. This entire process constitutes one cycle. The entire process continues repeatedly until the drug is completely extracted, a point when a solvent flowing from extraction chamber does not leave any residue behind. This method is suitable for plant material that is partially soluble in the chosen solvent and for plant materials with insoluble impurities. However, it is not a suitable method for thermolabile plant materials.

Evaporation:

Once the extraction process is complete, the round bottom flask with its content is removed and concentrated either using rotary evaporator or vacuum desiccator. This concentrated paste like material can be converted into powder form using lyophilizer.

Advantages. Large amount of drug can be extracted with smaller amount of solvent. It is also applicable to plant materials that are heat stable. No filtration is required, and high amount of heat could be applied.

***Disadvantages*:** Regular shaking is not possible, and the method is not suitable for thermolabile materials.

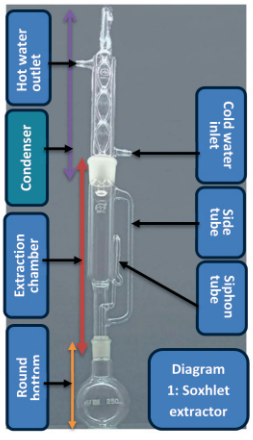

Diagram 1: Soxhlet extractor

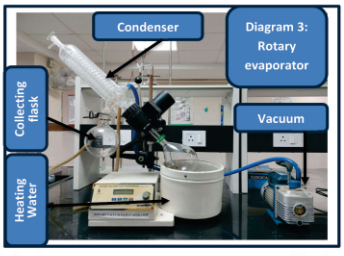

Diagram 3: Rotary evaporator

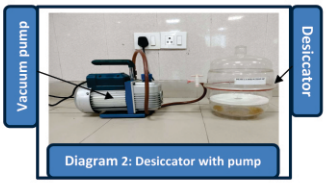

Diagram 2: Desiccator with pump

FRACTIONATION AND PURIFICATION METHODS

Fractionation is a process of separation of plant extracts into various fractions. The process continues until pure compound is isolated. When several solvents are

required for the fractionation, they should be added according to the order of increasing polarity. Fractionation techniques are basically classified into physical or chemical method.

A. Chemical methods

This extraction method is based on the type of functional groups possessed by a compound in the given mixture. Separation or purification can be achieved by chemical reactions using appropriate reagents.

B. Physical methods

Physical methods used in separation of compounds from mixtures include separation funnel method, chromatographic techniques, fractional distillation, fractional crystallization, fractional liberation, and sublimation.

IDENTIFICATION TECHNIQUES

Identification of compounds from medicinal plant extracts comprises detection of functional group, presence of multiple bonds and rings, hydrogen and carbon arrangement as well as full structural

elucidation. The techniques used include mass spectroscopy (MS), ultraviolet spectroscopy (UV), nuclear magnetic resonance spectroscopy (NMR), and infrared spectroscopy (IR).

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REGULATORY TOXICITIES STUDIES FOR IND APPLICATIONS

Prof. (Dr.) Bikash Medhi, Professor, Dept. of Pharmacology, PGIMER, Chandigarh

Regulatory toxicology studies for Investigational New Drug (IND) applications are essential to ensure the safety and efficacy of new pharmaceuticals before they are tested in humans. These studies involve a series of preclinical assessments, including pharmacology, pharmacokinetics, and toxicology evaluations, to understand the drug's behavior in the body and its potential effects on various biological systems.

The U.S. Food and Drug Administration (FDA) requires substantial evidence from these studies to determine that the drug is reasonably safe for initial human trials. The scope and duration of these studies depend on the nature and duration of the proposed clinical investigations. Typically, they include evaluations of the drug's effects on the cardiovascular, central nervous, and respiratory systems, among others. Compliance with Good Laboratory Practice (GLP) is generally expected for pivotal in vitro and in vivo studies submitted as part of the IND application.

Key nonclinical toxicology studies required for IND submission include assessments of acute, sub-chronic, and chronic toxicity, genotoxicity, and carcinogenicity. These studies help define the safety profile of the drug and identify any potential risks to patients. The data generated from these studies form the foundation for the IND application and are critical for obtaining approval to proceed with clinical trials.

In summary, regulatory toxicology studies for IND applications are a crucial step in the drug development process, providing essential safety data to protect patients and ensure the drug's potential efficacy. These studies help identify and mitigate risks, guiding the safe and ethical progression of new drugs into clinical testing.

Abstract Poster Presentation Session

Poster presentation

P-1

Evaluation of sedative drugs using cook's pole climbing apparatus

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Background: Sedative and anxiolytic drugs are widely used in clinical practice, and their efficacy can be evaluated using animal behavioral models. Cook's pole climbing apparatus assesses conditioned avoidance response in rodents and serves as a reliable tool to detect central nervous system (CNS) depressant activity.

Aim: The study aimed to evaluate and compare the sedative activity of selected drugs using Cook's pole climbing apparatus.

Methods: Thirty healthy albino rats were divided into three groups (n=10 each): Control (saline), Standard (diazepam 2 mg/kg), and Test drug (phenobarbitone sodium 15 mg/kg). Animals were conditioned to avoid electric shock by climbing the pole upon auditory and visual cues. After drug administration, the number of avoidance responses out of 10 trials were recorded per rat. Mean avoidance scores were compared among groups using one-way ANOVA followed by post-hoc test.

Results: Control animals showed a mean of 8.3 ± 0.8 successful avoidances per 10 trials, indicating intact conditioned response. Diazepam produced a significant reduction in avoidance behavior, with mean successful avoidances 2.1 ± 1.0 ($p<0.001$ vs control). The test drug showed moderate sedative effect, with mean avoidances 4.5 ± 1.2 ($p<0.01$ vs control; $p<0.05$ vs diazepam). This indicated that while the test drug impaired conditioned avoidance, its effect was less potent compared to diazepam.

Conclusion: Cook's pole climbing apparatus successfully demonstrated the sedative potential of test agents. The test drug reduced conditioned avoidance response significantly, though to a lesser extent than diazepam, suggesting partial CNS depressant activity. Further dose-response studies and clinical trials are needed to validate its sedative utility.

Keywords: Sedative drugs, Cook's pole climbing apparatus, conditioned avoidance response, diazepam, CNS depressant.

P-2

Demonstration of Screening of Analgesic Activity

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Background: Pain is one of the most common and distressing symptoms encountered in clinical practice, often leading to decreased quality of life and increased healthcare utilization. The management of pain relies heavily on the use of analgesics. Given the global rise in the use and misuse of these agents—particularly nonsteroidal anti-inflammatory drugs (NSAIDs), acetaminophen, and opioids—there is a growing demand for newer, safer, and more effective analgesic compounds. Effective screening methods are essential in the early stages of drug discovery to evaluate the analgesic potential of new compounds.

Objective: This workshop aims to demonstrate standard experimental models used in the screening of analgesic activity, providing both theoretical insights and practical exposure to both central and peripheral analgesic activity models.

Methodology: The study involved in vivo screening of analgesic activity using well-established animal models. Three primary methods were demonstrated: - the Hot Plate Method and Tail Flick Method, which assess central analgesic effects, and the Acetic Acid-Induced Writhing Test, which is used to evaluate peripheral analgesic mechanisms. Standard analgesic drugs like aspirin and morphine were used as controls to validate the models. The number of writhes and reaction time were recorded and compared using metrics like % inhibition and % maximum possible effect (%MPE).

Results: The standard drugs significantly reduced the number of writhes and increased reaction time in the respective models, confirming the reliability of the screening methods. Test compounds showed varying degrees

of analgesic activity, indicating the sensitivity of the models in detecting both peripheral and central analgesic effects.

Conclusion: The demonstration effectively illustrated the utility of preclinical models in screening for analgesic activity. These models are valuable tools in pharmacological research to identify and evaluate potential analgesic agents before clinical trials.

Keywords: Analgesic activity, Pain models, Acetic acid-induced writhing test, Hot plate method, tail flick method, Central analgesia, Peripheral analgesia, Preclinical screening, Efficacy evaluation, Laboratory demonstration.

P-3

Demonstration of screening of Anti-inflammatory activity

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PG Trainee[1], Tutor[2], Prof. and H.O.D[3]
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Introduction: Inflammation is a protective response of the body against infection, injury, or harmful stimuli. However, when it becomes uncontrolled, it can lead to chronic disorders such as arthritis, cardiovascular diseases, diabetes, and cancer. Anti-inflammatory drugs are commonly used, but their long-term use often causes side effects like gastric irritation, cardiovascular complications, and kidney damage. Hence, there is a growing interest in developing safer alternatives, particularly from natural products and phytoconstituents. Screening methods play a crucial role in identifying potential anti-inflammatory agents in the early stages of drug development.

Aim: The present study aims to demonstrate various screening methods for evaluating anti-inflammatory activity and to highlight their importance in identifying promising drug candidates.

Materials and Methods: Screening of anti-inflammatory activity can be carried out using both in-vivo and in-vitro models. In-vivo methods include carrageenan-induced paw edema, cotton pellet granuloma, and formalin-induced paw inflammation in laboratory animals such as albino guinea pigs and rats, which assess acute and chronic inflammation. In-vitro models involve assays like inhibition of protein denaturation, membrane stabilization, and nitric oxide scavenging activity, which evaluate the mechanism of drug action at the cellular or molecular level. Appropriate animal models, standard drugs for comparison, and test samples are selected to ensure reliability of results.

Results: Screening studies help determine whether a test compound possesses significant anti-inflammatory effects. Natural phytoconstituents and synthetic molecules tested through these models often show inhibition of inflammation, suggesting their potential as future drug leads. Results from in-vitro studies provide initial evidence, while in-vivo studies confirm efficacy and safety in living systems.

Conclusion: Demonstration of anti-inflammatory screening is an essential step in drug discovery. Proper selection of models and systematic evaluation can help in identifying new and safer anti-inflammatory agents, especially from natural sources. This approach can contribute to reducing the limitations of current therapies and advancing modern pharmacology.

Keywords: Inflammation, anti-inflammatory activity, screening models, phytoconstituents, drug discovery, in-vivo, in-vitro, animal models.

P-4

Study of muscle relaxant activity with the help of "rota rod apparatus"

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Background: Muscle relaxants are widely used in clinical practice for the management of spasticity, convulsi and as adjuvants in anesthesia. Evaluation of their central muscle relaxant activity can be carried out by animal models such as the rota rod apparatus, which measures the ability of rodents to maintain balance rotating rod.

Objectives: To assess the muscle relaxant potential of the test drug(s) by comparing their effects on motor coordination in rodents using the rota rod test.
Materials and Methods: This experimental study was conducted on healthy Swiss albino mice weighing between 20 and 25 g, which were randomly divided into three groups of six animals each. Group I served as the control and received normal saline, Group II served as the standard and was administered diazepam at a dose of 2 mg/kg intraperitoneally, and Group III received the investigational compound at a dose of 10 mg/kg orally. The rota rod apparatus, maintained at a constant speed of 20 rpm, was employed to evaluate motor coordination, and prior to drug administration, all animals were trained to stay on the rotating rod for 180 seconds to ensure baseline performance. Following administration of the respective treatments, the fall-off time of each animal from the rotating rod was recorded at intervals of 30, 60, and 120 minutes.
Results: In the control group, mice were able to maintain balance on the rod with a mean fall-off time of 165 ± 10.5 seconds, indicating no significant motor incoordination. In contrast, the diazepam-treated group demonstrated marked muscle relaxation with a significant reduction in fall-off time to 35 ± 8.2 seconds at 60 minutes when compared to the control group ($p < 0.001$). The investigational drug group exhibited moderate muscle relaxation with a fall-off time reduced to 92 ± 12.6 seconds at 60 minutes, which was statistically significant in comparison with the control group ($p < 0.05$), though its effect was notably less potent than diazepam ($p < 0.01$).
Conclusion:
The test compound exhibited significant central muscle relaxant activity in mice as assessed by the rota rod test, though its effect was less pronounced compared to the standard diazepam. These findings suggest that the investigational drug possesses moderate muscle relaxant potential and may warrant further pharmacological evaluation.
Keywords: Muscle relaxant activity, Rota rod apparatus, Motor coordination, Diazepam, Experimental pharmacology, mice, CNS depressant

P-5

Demonstration of screening of effect of drugs on wound healing of Rat

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Introduction: *Telephium imperati* (L.), a plant from the Caryophyllaceae family, is traditionally used in Morocco for wound healing. Its medicinal effects are believed to be due to its bioactive compounds. However, scientific evidence for its wound healing potential is limited.

Aim: This study aimed to evaluate the wound healing activity of a 5% hydroalcoholic extract ointment of *Telephium imperati* (L.) on skin burn wounds in Wistar rats.

Materials and Methods: Phytochemical screening and thin-layer chromatography (TLC) were performed to identify active compounds in the plant extract. Eighteen healthy male Wistar albino rats (150–180 g) were divided into three groups (n=6):

- **Test group:** Treated with 5% *Telephium imperati* extract ointment.
- **Control group:** Treated with Vaseline.
- **Reference group:** Treated with standard drug (MADECASSOL®).

Burn wounds were created on the dorsal area of each rat. Wound areas were measured every 5 days for 55 days. Histopathological analysis was performed 24 h after burn creation, and on days 15, 25, and 55.

Results: Phytochemical analysis revealed the presence of saponins, flavonoids, and quercetin. By the end of the experiment, wound contraction was 95.5% in the test group, 97.5% in the reference group, and 75.75% in the

control group. Histology of the test group showed strong fibroblast proliferation, minimal inflammatory cells, and well-organized collagen, indicating faster and better wound healing compared to control.

Conclusion: The 5% hydroalcoholic extract ointment of *Telephium imperati* significantly enhanced burn wound healing in rats, supporting its traditional use in Moroccan medicine. The plant's bioactive compounds, especially flavonoids and quercetin, may contribute to its healing properties.

Keywords: *Telephium imperati*, wound healing, hydroalcoholic extract, burn wound, Wistar rats, phytochemicals.

P-6

The impact of animal handling on research quality and ethical compliance

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Introduction: In clinical pharmacology and experimental physiological research, animal handling is a vital part. Irregular handling imposes stress, changing physiology, hormones, and behaviour, which may affect drug metabolism like absorption, response and elimination. In India, a committee named the CPSCEA (Committee for the Control and Supervision of Experiments on Animals) governs the humane handling and application of replacement, reduction, and refinement.

Methods: This commentary is based on CPCSEA guidelines and international standards, such as ARRIVE (Animal Research: Reporting of In Vivo Experiments) and OECD (Organisation for Economic Co-operation and Development Test Guidelines), as well as PubMed-indexed studies. It outlines the effects of handling stress markers and pharmacological outcomes, and emphasises the importance of training and refinement of procedures.

Discussion: Relevant studies revealed that handling of animals increases corticosterone and anxiety, while tunnel or cupped (hand) handling reduces stress and enhances animal-human interaction [1,2]. This stress can alter or decrease immunity, cardiovascular response, and have an effect on drug metabolism, leading to unreliable results [3]. In India, as per CPCSEA guidelines, staff are required to receive training in species-specific methods, constant supervision and adaptation of enhancements such as habituation to the surroundings, decreased restraints and non-invasive techniques to ensure ethical and scientific standards [4,5].

Conclusion: The attention of animals is not merely a mechanical practice; rather, it is a factor that affects both the ethical and scientific accuracy of the research. Decreasing stress, safeguarding the reproducibility of data, and confirming the reliability of pharmacological research are all outcomes that can be accomplished by following CPCSEA recommendations in India, in addition to world best practices.

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P-7

Protective role of natural antioxidants against drug-induced hepatotoxicity (paracetamol, isoniazid)

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Background: Drug-induced hepatotoxicity is a common clinical problem, with paracetamol and isoniazid being important causes of acute and subacute liver injury. Oxidative stress and lipid peroxidation play a central role in

the pathogenesis. Natural antioxidants such as vitamin C may provide protection by scavenging free radicals, enhancing endogenous defense systems, and stabilizing hepatocyte membranes.

Aim: To evaluate the protective role of vitamin C against paracetamol- and isoniazid-induced hepatotoxicity in experimental rats.

Materials and Methods: An experimental study was conducted in the Department of Pharmacology, JLNMC, Bhagalpur, using healthy Wistar albino rats (150–200 g). The animals were divided into three groups (n = 6 each): Group I (control, vehicle only), Group II (toxic control, hepatotoxic dose of paracetamol 2 g/kg single oral dose or isoniazid 50 mg/kg/day orally for 21 days), and Group III (treatment, vitamin C 100 mg/kg/day orally along with hepatotoxic drug). At the end of the experiment, blood samples were analyzed for biochemical markers (ALT, AST, ALP, total bilirubin) and liver tissue homogenates were assessed for oxidative stress marker malondialdehyde (MDA). Data were analyzed using one-way ANOVA with Tukey's post hoc test.

Results: The toxic control group showed significant elevation of ALT, AST, ALP, total bilirubin, and MDA compared with controls ($p < 0.001$). Co-administration of vitamin C significantly reduced these parameters compared with the toxic group ($p < 0.001$), though values did not completely normalize.

Conclusion: Vitamin C exhibits significant hepatoprotective activity against paracetamol- and isoniazid-induced hepatotoxicity, primarily through its antioxidant effect in reducing lipid peroxidation. These findings support further clinical evaluation of natural antioxidants as adjuncts in preventing drug-induced liver injury.

Keywords: Hepatotoxicity, paracetamol, isoniazid, vitamin C, oxidative stress, malondialdehyde

P-8

Antidepressant-like activity of omega-3 fatty acids in chronic mild stress model of depression in rats

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Background: Depression is a multifactorial psychiatric disorder associated with neurochemical, inflammatory, and oxidative imbalances. Omega-3 fatty acids, particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are essential polyunsaturated fatty acids known to modulate neuronal membrane fluidity, neurotransmission, and neuroinflammatory pathways. Emerging evidence suggests their potential antidepressant effect through serotonergic and anti-inflammatory mechanisms.

Aim: To evaluate the antidepressant-like activity of omega-3 fatty acids in rats exposed to a chronic mild stress (CMS) model of depression.

Materials and Methods: Thirty healthy adult Wistar rats (150–200 g) were randomly divided into three groups (n=10 per group):

- **Group I:** Normal control (no stress, received distilled water)
- **Group II:** CMS control (subjected to 28-day mild stress protocol)
- **Group III:** CMS + Omega-3 fatty acids (100 mg/kg/day, orally for 28 days)

Depression was induced using the CMS paradigm involving variable stressors (restraint, cold exposure, overnight illumination, wet bedding, etc.). Behavioral assessments were performed using the Forced Swim Test (FST) and Sucrose Preference Test (SPT). Biochemical estimation of brain malondialdehyde (MDA), superoxide dismutase (SOD), and serotonin (5-HT) levels were also conducted. Data were analyzed using one-way ANOVA followed by Tukey's post hoc test, with $p<0.05$ considered statistically significant.

Results: Omega-3-treated rats demonstrated a significant decrease in immobility time in FST ($p<0.01$) and a significant increase in sucrose preference ($p<0.05$) compared to CMS controls. Biochemically, omega-3 supplementation significantly reduced MDA levels and enhanced SOD and 5-HT concentrations in brain tissue, indicating improved antioxidant status and serotonergic activity.

Conclusion: Omega-3 fatty acids exhibit marked antidepressant-like activity in rats subjected to chronic mild stress, likely mediated through modulation of oxidative stress and serotonergic neurotransmission. These findings support the therapeutic potential of omega-3 fatty acids as a safe adjunctive strategy in the management of depression.

Keywords: Omega-3 fatty acids, Chronic mild stress, Depression, Forced swim test, Serotonin, Antioxidant.

P-9

Design of Animal Experiments in Pharmacological Research

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Background: Pharmacology is the scientific study of the effects of drug & chemical on living organism it also known as pharmaceutical science, has made significant progress, especially in the 20th century, and played a fundamental role in the development of modern medicine. Pharmacology uses in vitro, in vivo, and clinical research in drug development. Experimental animals are more important in in vivo research. The majority of the drugs used today were developed from animal research. In animals experimental in pharmacological research will be used should be planned carefully, and a minimum number of animals should be used since the subject is a living being. One of the most important ethical principles is to avoid procedures that may cause unnecessary torture and pain to animals during experiments.

Aim: The Aim of pharmacological research is to develop drugs for the treatment or diagnosis of diseases. It is aimed at determining the effects of the substance you are researching in the presence of disease.

Results and Conclusions: Immediate use of substance whose effects were previously unknown on humans may lead to various adverse effect & even death. After many adverse events in the past, drug development stages have been determined by accepted international rules. According to these rules, the effect of the substance being investigated must be investigated in experimental animals that have been used as disease models before humans. Many disease models have been developed for this purpose. Drugs developed in these disease models created in experimental animals are now successfully used in the treatment of humans.

Abstract Oral Presentation Session

Oral presentation

O-1

Systematic review of adverse effects of GLP-1 receptor agonist in obese adults

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Background: Glucagon-like peptide-1 receptor agonists (GLP-1 RAs) are increasingly used for obesity management, but their safety profile in non-diabetic and diabetic adults with obesity requires further studies and experimentation.

Methods: We searched PubMed, google scholar, science direct for open articles published 2020 2025 reporting any GLP-1 RA was used to treat obesity in adults aged 18–60. Inclusion criteria were: original RCTs, non-randomised, clinical trials, or prospective/retrospective cohort studies; participants with obesity (obese according to WHO criteria of BMI>30); GLP-1 RA exposure; and report of adverse events. Exclusion criteria were studies limited to pregnancy, paediatric populations (<18 years), meta-analyses, systematic reviews, narrative reviews, case reports/series, animal studies and studies without extractable adverse-event data. Two reviewers independently screened titles/abstracts and full texts, extracted data, and assessed risk of bias using RoB 2 for RCTs and the Newcastle–Ottawa Scale for observational studies. Pre-specified safety outcomes included overall adverse events, gastrointestinal events (nausea, vomiting, diarrhoeas, constipation), pancreatobiliary events (pancreatitis, gallbladder disease), Dermatological event, cardiovascular events, hypoglycaemia, neuropsychiatric events (mood changes, anxiety, depression, insomnia), injection-site reactions, and treatment discontinuation due to adverse effects.

Analysis: Where clinical and methodological heterogeneity permitted, we planned random-effects meta-analyses to estimate pooled incidence rates and risk ratios; heterogeneity will be assessed with I^2. If pooling was inappropriate, results will be synthesised narratively with tabulated study-level data. Sensitivity analyses will explore effects by specific agent, dose, treatment duration, and diabetic status. Overall certainty of evidence will be graded using GRADE.

Conclusions: This systematic analysis will provide an up-to-date synthesis (2020–2025) of adverse events associated with GLP-1 RAs in adults with obesity, informing clinicians and patients about the relative safety profile across different drugs, doses, and treatment durations

O-2

Drug-based therapies in POEMS syndrome: pharmacologic mechanisms, adverse effects, and challenges in India and worldwide

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Introduction: POEMS syndrome is a rare multisystem disorder driven by plasma-cell dyscrasia and vascular endothelial growth factor (VEGF) overproduction. Autologous stem cell transplant (ASCT) is highly effective but not curative, as relapse and progression are seen in many patients. Moreover, a substantial proportion are ineligible for ASCT and rely on pharmacotherapy. However, evidence on drug mechanisms, adverse effects, and real world applicability remains scattered, especially in India. Rationale: To our knowledge, this is the first review from a pharmacology perspective that synthesises therapeutic mechanisms, safety concerns, and contextual challenges of drug-based management in POEMS. Prior literature has largely emphasised clinical outcomes without dedicated focus on pharmacologic toxicity or India-specific data, creating an unmet need.

Objectives:

1. To summarise pharmacological treatments in POEMS and their mechanisms of action.
2. To evaluate reported adverse effects and their pharmacologic basis.

3. To map Indian evidence against global experience, highlighting safety and accessibility. Inclusion/Exclusion: **Included:** all primary reports (case reports, series, cohorts, and clinical trials including the J-POST RCT) describing drug therapy and adverse effects.

Excluded: studies focusing solely on ASCT or radiotherapy. Methods: PubMed, Scopus, and ClinicalTrials.gov were searched to September 2025 using "POEMS" with drug-specific terms. Two reviewers independently screened eligible studies and synthesised findings, following PRISMA principles adapted for rare disease literature.

Results: Evidence suggests immunomodulatory drugs (thalidomide, lenalidomide) and proteasome inhibitors yield clinical and neurological responses but might result in toxicity. Anti-VEGF therapy lowers VEGF rapidly but has caused severe, fatal complications in two cases. Indian literature is confined to single-centre reports, with under-reporting of adverse events.

Conclusion: This pharmacology-focused synthesis highlights therapeutic mechanisms, identifies major safety issues, and underscores the need for structured adverse-event reporting and region and patient specific treatment strategies for ASCT-ineligible patients with POEMS.

Keywords: Bortezomib, Bevacizumab, Crow-Fukase syndrome

O-3

Impact of 2023 CPCSEA Guideline Revisions on the Adoption of In-vitro and In-silico Methods in Indian Pharmacology Research: A 5-Year Trend Analysis (2020–2025)

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Introduction: Animal experiments have long been central to preclinical pharmacology research in India. Ethical concerns and evolving regulations have prompted a shift toward alternative approaches. The CPCSEA revised its guidelines in 2023 to emphasize the 3Rs (Replacement, Reduction, Refinement) and promote New Approach Methodologies (NAMs), including in-vitro and in-silico techniques.

Background: NAMs offer improved human relevance and reduced ethical burden, yet their uptake in Indian pharmacology has been variable. Evaluating recent trends provides insight into the impact of regulatory changes and research adaptation.

Material & Methods: A bibliometric observational study was conducted using PubMed, Scopus, and selected Indian pharmacology journals (*Indian Journal of Pharmacology*, *Journal of Pharmacology & Pharmacotherapeutics*). Original research articles with at least one India-affiliated author published from January 2020 to September 2025 were included. Articles were categorized as animal-based, in-vitro, in-silico, or mixed. Annual publication counts and proportions were calculated, and trends analyzed using Poisson regression and segmented regression to compare pre- (2020–2022) and post-guideline (2023–2025) periods.

Results: NAM-related publications showed a consistent increase over the five-year period, with a sharper rise after the 2023 CPCSEA guideline revisions. In-vitro studies expanded in drug screening and toxicology, whereas in-silico studies grew in pharmacokinetic modeling and drug repurposing. Animal-based studies remained dominant but showed a relative decline. Leading contributions originated from central universities and national research institutes.

Conclusion: The 2023 CPCSEA guideline revisions appear to have accelerated the adoption of NAMs in Indian pharmacology research. The increasing shift toward in-vitro and in-silico approaches reflects a trend toward ethical research practices and improved translational relevance.

Keywords: CPCSEA, New Approach Methodologies (NAMs), In-vitro, In-silico, 3Rs

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