Name of Unit	Research Methodology and Bio-Statistics
Subject /Course name	Experimental Pharmacology
Subject/Course ID	BP 810ET
Class: B.Pharm. Semester	VIII
Course coordinator	Ms. Medha Bhalla
Mobile No.	9478951265
Email id	Medhabhalla2103@gmail.com

Learning Outcome of Module 05

LO	Learning Outcome	Course Outcome Code
LO 1	To remember and understand the basic concepts of research methodology, including selection of research topic, literature review, research hypothesis, and study design.	BP810.6
LO 2	To apply statistical tools such as Student's t-test and One- way ANOVA for analysis of preclinical data.	BP810.6
LO 3	03 To analyze and interpret experimental data obtained from pharmacological studies.	BP810.6
LO 4	To create graphical representations of research data and evaluate the results for scientific interpretation.	BP810.6

Content Table

Topic
• Selection of research topic • Review of literature • Research hypothesis • Study design. • Pre-clinical data analysis and interpretation using Students't' test. • One-way ANOVA. • Graphical representation of data.

BASICS OF RESEARCH

Basic idea of research methods and methodology is described with special emphasis on pharmacological studies.

Definition: Many definitions are put forward to define the term "research". In common terms it refers to a search for knowledge. Moreover, it can also be defined as a scientific and systematic search for relevant information on a specific topic. In addition, it is also called as art of scientific investigation, while the dictionary meaning of research is "a careful investigation or inquiry especially through search for new facts in any branch of knowledge.

Thus, it is a systematic way to answer the unknown or unanswered questions. Difference between Research Methods and Research Methodology: It is important to note that all those methods/techniques that are used by a researcher for conducting of research are called as research methods.

On the other hand, the ways used to systematically solve the research problem is called as Research methodology. Thus, in other words, research methodology is a science of studying how research is done scientifically. Therefore, for a researcher to conduct any type of research, he should be aware of both research methods and research methodologies.

TYPES OF RESEARCH

Many scientists have classified research in various categories. The following section describes only the basic types of research:

(i) **Descriptive versus Analytical:**

Descriptive research includes surveys/scientific reviews and fact-finding enquiries different kinds. The major purpose of descriptive research is compiling of the state scientific or general affairs, as it exists at present. The main characteristic of Ex post facto studies is that the researcher can only report what has happened or what is happening with no control over the variables. The methods of research utilized in descriptive research are survey methods of all kinds including scientific reviews comprising of both comparative and co-relational methods. On the other hand, in analytical research, the researcher uses scientific facts or information already available, and analyze these to make a critical evaluation of the scientific material. Therefore, the descriptive research is only used to compile the status of a particular scientific field while, using the current information, critical evaluation of the compiled scientific material can be done by the researcher in analytical research.

(ii) **Applied versus Fundamental**:

Research can either be applied (or action) research or fundamental (to basic or pure) research. Applied research aims at finding a solution for an immediate or existing problem facing a society or an industrial/business organisation like discovering a new pharmacological agent for the treatment of an epidemic. On the other hand, fundamental research is mainly concerned with generalisations and with the formulation of a theory i.e. as "Gathering knowledge for knowledge's sake is termed as 'pure' or 'basic' research." Determining the role of a neurotransmitter in the working of any system e.g. CVS, CNS or ANS etc. is an example of fundamental research. Similarly, research aimed at certain solution facing a concrete problem like development of new drug or new device the treatment of a disease is an example of applied research. Therefore, the applied research is aimed to discover a solution for some existing practical problem, while basic research adds to the already existing organized body of scientific knowledge.

(iii) **Quantitative versus Qualitative:**

Quantitative research is based on the measurement of quantity or amount. On the other hand, qualitative research, on the other hand, is concerned with qualitative phenomenon. For example, when we investigate the effect of analgesic activity of a test drug on humans and if they are asked they felt any relief from pain. Then the answers of such clinical data were in the aim is to discover the underlying effect on human behaviour is qualitative research. Thus, qualitative research is the research in which the data cannot be quantified but it is of qualitative nature. This type of research aims at discovering the underlying motives and desires, using in depth interviews for the purpose. On the contrary, in quantitative research, the data can be measured for example, effect of acetylcholine (Ach) on the contraction of ileum. In such a case, the length of the contraction induced by Ach in ileum can be measured and is reported as percentage response. Thus, the research in which the data can be quantified is called as quantitative research.

(iv) **Conceptual versus Empirical:** Research related to some abstract idea(s) or theory is called as conceptual research. It is generally used by philosophers and thinkers to develop new concepts or to reinterpret existing ones example Einstein theory of "relativity". This type of research can also be called as thought research. On the other hand, empirical research is data-based research experimental type of research in which, researcher comes to conclusion that can be verified by observation or experiment. However, a working hypothesis or guess as to the probable.

results is firstly proposed by the researcher and then sets up experimental designs address the hypothesis. Thereafter, experimental data is generated by him to prove disprove his hypothesis, wherein the experimenter's have control over the variables under study. Empirical research is today considered to be the most powerful way to ratify a hypothesis.

(v) **Other Types of Research**:

All the following types of research basically belong to the above main types with different variables:

1. **One-time research or longitudinal research.**

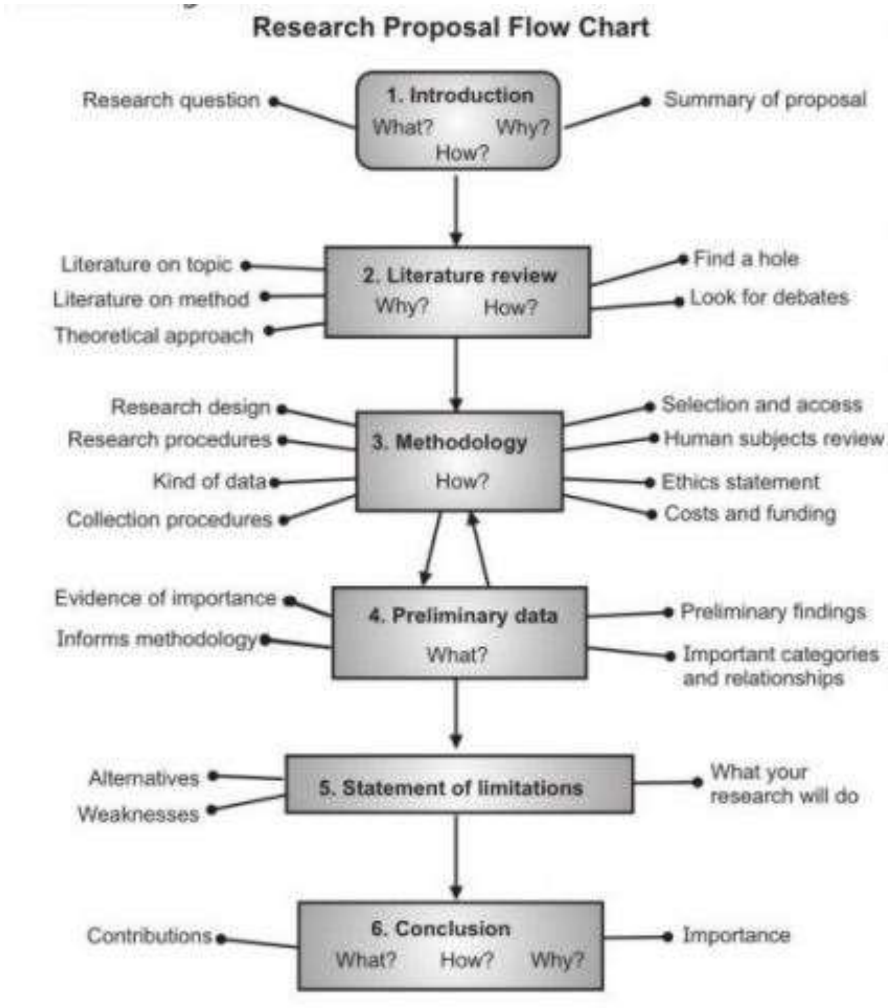
2. Field-setting research or laboratory research or simulation research

3. Clinical or diagnostic research.

4. Historical research

5. Research can also be classified as conclusion-oriented and decision-oriented.

After understanding the definition and types of research methods, it important to delineate methodologies or the systemic path by which the research needs to be conducted.



RESEARCH PROCESS

Selection of Research Topic

Research process consists of a series of steps or actions required for effectively conducting research. The following are the steps that provide useful procedural guidelines regarding the conduct of research:

(1) Introduction: Selection of research problem/question.

(2) Extensive literature survey: Developing hypothesis.

(3) Research methodologies: Preparing the research design and ethics clearance.

(4) Preliminary data: Execution of the project, generating results and analysis of data.

(5) Statement of limitations: Hypothesis testing, generalization and interpretation with available literature.

(6) Conclusions: Preparation of the report or presentation of the results and contribution of results to the field of research.

STEP ONE: Selection of research topic/question: A research problem/question/topic refers to an unanswered question that a researcher might encounter in the context of either a theoretical or practical situation, which he/she would like to answer or find a solution to. Thus, the first stage in the research process is to select and properly define the research question. A researcher should be able to first identify a problem and formulate it to conduct research on that topic. Journalists often use a basic formula for writing i.e. 5 Ws and an H structure:

Who? What? Where? When? Why? and How?

Researcher using the 5 Ws and an H formula can plan research, although can consider them in a different order. In selecting a research topic/question generally the research should start with following step: What? i.e. What do I want to know? the Thus, to plan a research it is important for the researcher to properly understand and clarify what it is he wants to know. After identifying and understanding What?- researcher can call it as the research question/problem/topic on which he can work. Thus, after this step researcher will be able to answer all of the other two questions that are necessary to plan research properly that will help to decide to continue with the research topic, such as:

How? - How do I find out what I want to know? And why?

Finding the right answers to the research question. After this, research question/topic can be exactly formulated and then the researcher can proceed to the next step for i.e. review of literature. As such, the general literature survey begins while selecting the research topic but in the second step of review of literature is conducted especially on the research topic.

STEP TWO:

Review of literature and Research hypothesis:

Review of literature

Selection of Research Topic

After selection or identification of research question or topic, the second step of research proposal begins i.e. topic related review of literature. The main aim of the review of literature is to find out the answers to perfectly design the experiment of research proposal. The following information is gathered from various sources for rightful execution of the research topic:

1. **National and international status of the research topic**: Literature on the theoretical part of the topic is conducted to identify the loopholes and debates by the past and recent research reports related to the research problem. This will help the researcher to decide the preliminary hypothesis on which he can further design the research protocols.

2. **Methodological review of literature**: After putting forward the preliminary hypothesis, the researcher should conduct literature survey on the methodological part i.e. how? the aim (hypothesis) will be achieved and what? methods need to be followed and performed to answer all the questions of the research questions. This literature survey will help to decide the objectives of the study to achieve the aim or ratify the hypothesis of the research topic. In order to conduct the review of literature following approaches are recommended:

Conducting a local/background information scan:

Theoretical survey: After deciding the research question (or questions) a local information scan should be conducted by surfing on the Internet, or ask a few experts in the field you are researching, or perhaps you need to speak to a few people in the community. This will help researcher to know what information is already available at national level and also help him to refine the research question. Moreover, it is possible that some or all aspect of the selected research topic has already been done. In such a case, research may decide for a different kind of question, or focus on specific areas of research. After conducting a local information scan, researcher can take another look at the research question to decide whether to change it or refine in any way? Therefore, after constructing the research question or questions, researcher can decide quite specifically what it is he want to know. These kinds of information will also help in the interpretation of the research results and finally to come to a decision or a number of decisions.

Literature review tip

There are three possible sources of information for research: 1. Empirical sources: gathering information from the real world. This may be primary data that you have gathered yourself, or secondary data gathered by someone else - 2 3. e.g. published statistics or company documents. Literary sources: gathering information from published books and papers, and from the internet (Stein, 1999). Conceptual analysis: analysing the meanings of concepts and their implications. (Mathematical analysis and model building are conceptual in that they concerned with working out the detailed implications of assumptions. In general, some of the direct sources are listed below:

Internet (general searching/specific websites)

E-journal

Hardbound journal

Pamphlets/promotional material

Conferences

Experts (you may want to develop a database of experts in various fields)

Magazines

E-newsletters

Keeping track of published research (usually by signing up to mailing lists or e-newsletters, but keep an eye on the media as well; a lot of research - especially social research - will be written about in newspapers and magazines). Direct email contact with the experts in the field.

Lots of information

Use selected key words: However, a lot of background information on the issue you are researching present using the above ways. Therefore, research need to keep his research question clearly in mind and decide as you go along whether the information you have found is relevant to your research needs. For this purpose, it is recommended that proper key words should be used while searching the literature especially on internet.

Save the information

It is also recommended that researcher should record or store the information collected in a systematic way. For example, the information obtained from book should be stored with details such as title, publisher, date/year of publication, place of publication and authors' names, or you may want to bookmark the relevant websites you find on your Internet browser. Moreover, the research article information should be stored with details such as title, publisher, date/year of publication, place of publication if any and authors' names, volume number, issue number and page number or you may save the URL of the relevant websites you find on your Internet browser. All this process is important because the literature information obtained during the survey will be needed to generate the list of reference and acknowledging the source/author from where the information was collected during the compilation of data and interpretation. Moreover, referencing is a time consuming process and it is possible that researcher might forget the source of a quote or idea during the execution of research proposal. Some softwares are now a days available like Endnote and Reference manager using which, the referencing can be done easily.

Research Hypothesis

In general "Hypothesis may be defined as a proposition or a set of propositions set forth as an explanation for the occurrence of some specified group of phenomena either asserted merely as a provisional conjecture to guide some investigation in the light of established facts.

When adopting scientific methods a prediction or a hypothesized relationship is tested then it is known as research hypothesis. The research hypothesis is a predictive statement, which relates to a dependent variable and an independent variable. Generally, a research hypothesis must consist of at least one dependent variable and one independent variable. For instance, the following statements may be considered:

- **"Students who take tuitions perform better than the others who do not receive tuitions" or,**
- **"The female students perform as well as the male students". These two statements are hypotheses that can be objectively verified and tested.**

Thus, they indicate that a hypothesis states what one is looking for. Besides, it is a proposition that can be put to test in order to examine its validity.

Characteristics of Hypothesis

A hypothesis should have the following characteristic features:

- A hypothesis must be precise and clear.
- A hypothesis must be capable of being put to test. Therefore, the researcher may
- conduct some prior study in order to make a hypothesis testable. (Kothari, 1985). A hypothesis must state relationship between two variables, in the case of relational hypotheses.

- A hypothesis must be specific and limited in scope.
- As far as possible, a hypothesis must be stated in the simplest language, so as to make it understood by all concerned.
- A hypothesis must be consistent and derived from the most known facts.
- A hypothesis should state the facts that give rise to the necessity of looking for an explanation. Testing of hypotheses requires a researcher to be familiar with various concepts concerned with it such as: Null Hypothesis and Alternative Hypothesis (to be discussed with statistics).

STEP THREE:

Research Methodologies i.e. to find out how the study should conducted? be After conducting the literature review, the preliminary hypothesis is decided and then researcher can formulate the exact aim and objective/s of the research so that hypothesis can be proved. Generally, using the information obtained during the background research, the methodological review of literature should also be conducted to exactly identify the experimental methods and procedure that needs to be followed to test the aim and objective.

The following points are generally taken in consideration during pharmacological screening methodologies:

- **Research/study design.**
- **Experimental and surgical procedures to be performed.**
- **Type of data that will be collected.**
- **Procedures for collecting data.**
- **Instruments required.**
- **Subject: animals and human.**
- **Chemicals and glassware.**
- **Selection of Research Topic**
- **8. Ethical clearance for animal/human study.**
- **Total cost of the research project.**

At this point, you may want to build a simple table, setting out the most appropriate methodologies, and where you are going to find the information, as well as some of the challenges you may have to overcome.

Study/Experimental design

The main component of research methodologies section is study/experimental design. The experimental design depends on the objectives of the study. It should be planned in detail, including the development of written protocols and consideration of the statistical methods to be used, before starting work. In principle, a well-designed experiment avoids bias and is sufficiently powerful to be able to detect effects likely to be of biological importance. It should not be so complicated that mistakes are made in its execution. The study design is also called as research design and can be defined as the arrangement of conditions for collection and analysis of data in a manner that aims to combine relevance to the research purpose with economy in procedure. Infact, research design is the conceptual structure within which research is conducted; it constitutes the blueprint for the collection, measurement and analysis of data (Selltiz et al, 1962). Thus, research design provides an outline of what the researcher is going to do in terms of framing the hypothesis, its operational implications and the final data analysis. Types of research design

- **There are different types of research designs and are broadly categorized as:**
- **Exploratory Research Design.**
- **Descriptive and Diagnostic Research Design.**
- **Hypothesis-Testing Research Design.**

Exploratory Research Design

The major purpose of such design is the discovery of ideas and insights. The exploratory research design is also known as formulative research design as it formulates a research problem for an in-depth or more mor precise investigation, or for developing a working hypothesis from an operational aspect. Research design of this type should be with in-built flexibility as the initial research problem would be transformed into a more precise one in the exploratory study.

Descriptive and diagnostic research design

The experimental work conducted to describe the characteristics of a particular individual or a group and then it is a descriptive research type of design. On the other hand, the study that analyzes whether a certain variable is associated with another it is called as diagnostic research study. Generally, most of the social research design falls under category

3. **Hypothesis testing research design:**

Selection of Research Topic,

The Hypothesis-Testing Research Designs are those in which the researcher tests hypothesis of causal relationship between two or more variables. These studies require procedures that would not only decrease bias and enhance reliability, but also facilitate deriving inferences about the causality. Generally, experiments satisfy such requirements. Hence, when research design is discussed in such studies, it often refers to the design of experiments. In the pharmacological screening, virtually all animals experiment/research design should be performed considering the following points

1. **Experimental Unit**
2. **Randomization**
3. **Blinding**
4. **Pilot Studies**
5. **Formal Experimental Designs**

(a) Completely randomized

(b) Randomized block

(c) Factorial designs

(d) Latin square, crossover

(e) Repeated measures

(f) Split-plot

(g) Incomplete block (h) Sequential designs

6. **Dependent Variable(s)**
7. **Independent Variables or Treatments**
8. **Uncontrolled (Random) Variables**
9. **Ethical approval**

Experimental Unit:

Each experiment involves a number of experimental units, which can be assigned at random to a treatment. The experimental unit should also be the unit of statistical analysis. For example, if caffeine, a CNS stimulant is treated to all animals in the same cage and vehicle is given to animals in another cage therefore the cage of animals (not the individual animals within the cage) is the experimental unit and can be assigned as caffeine treated group and vehicle treated group. However, in some case individually animals in one cage can also become experimental unit if each is treated with different treatments. In such case, the animals are need to be given identification mark like numbering to 1, 2 and 3, or X, Y and Z on its tail to identify the each animals as experimental unit (for the treatment given). Such type of design is called as crossover experimental design.

Split-plot experimental designs have more than one type of experimental unit. For example, cages each containing two mice could be assigned at random to a number of food treatments (so the cage is the experimental unit for comparing foods), and the mice within the cage may be given one of two different treatments by injection (so the mice are experimental units for the treatments effect). In each case, the analysis should reflect the way the randomization was done.

2. **Randomization:**

Treatments should be assigned so that each experimental unit has a known, often equal, probability of receiving a given treatment. In simple words, each animal or group of animals are not be assigned to a treatment or experimental unit [n = 5-8] predetermined, but are chosen on lottery basis, this process is termed as randomization. Alternatively, treatment assignments can be written on pieces of paper and drawn out of a bag or bowl for each experimental unit (e.g., animal or cage). Randomization is essential to avoid the sources of variation, known or unknown, which could bias the results. Note that the different treatment groups should be processed identically throughout the whole experiment i.e. measurements, treatment, handling of animals, time of administration, number of treatments and most importantly, animals of different treatment groups should not be housed on different shelves or in different rooms because the environments may affect the treatment outcome.

3. **Blinding:**

To avoid biasness of the researcher who have designed the experiments to bring the expected outcome, all the experiments should be performed "blind" by a trained person who is unaware of the treatment given to each experimental unit. For this purpose, after the randomized of animals (or other experimental unit) to a treatment group; the treatments, animals, samples, and treatments should be coded until the data are analyzed.

4**. Pilot Studies**

It is advisable that before conducting large-scale experiments on animal, pilot studies should be preformed to test the logistics of a proposed experiment using only a single animal or slightly larger group. This pilot studies will provide estimates of the means, standard deviations and also some indication of likely response that can be used in a power analysis to determine sample sizes of future experiments. However, pilot experiment with very small group might give inaccurate estimate.

5. **Formal Experimental Designs**

Literatures have described several formal experimental designs but the most common adopted are completely randomized, randomized block, and factorial designs. However, Latin square, crossover, repeated measures, split-plot, incomplete block, and sequential designs are also used. The special features and constraints of the experimental material and the nature of the investigation are taken in account while developing these formal designs. It is not possible to describe all of the available experimental designs but the first three designs, which are commonly used are explained here.

(a) **Completely Randomized Designs:**

In which animals (or other experimental units) are assigned to treatments at random, are widely used for animal experiments. The main advantages are simplicity and tolerance of unequal numbers in each group, although balanced numbers are less important now that good statistical software is available for analyzing more complex designs with unequal numbers in each group. However, simple randomization cannot take account of heterogeneity of experimental material or variation (e.g., due to biological rhythms or environment), which cannot be controlled over a period of time.

(b) **Randomized block**:

This type of experimental design is used to split an experiment into a number of "miniexperiments" to increase precision and/or take account of some natural structure of the experimental material. It is important to note that during large experiments treatment of all the animals at the same time or house them in the same environment is extremely difficult. Thus, in such case, it may be better to divide the experiment into smaller blocks that can be handled separately. A "block" will consist of one or more animals (or other experimental units) that have been assigned at random to each of the different treatment groups. Blocking thus ensures balance of treatments across the variability represented by the blocks. However, in situation like when the experimental animals differ excessively in age or weight, it may be best to choose several groups of uniform animals and then assign them to the treatments within the groups. These type of block designs are more powerful than completely randomized designs, but their benefits depend on correct analysis, using (usually) a two-way ANOVA without interaction.

(c) **Factorial designs:**

Factorial experiments have more than one type of treatment or independent variable (e.g., a drug treatment and the sex of the animals). The aim could be to learn whether there is a response to a drug and whether it is same in both sexes (i.e., whether the factors interact with or potentiate each other). These designs are often extremely powerful in that they usually provide more information for a given size of experiment than most single factor designs at the cost of increased complexity in the statistical analysis. They are described in most statistical texts (Cox, 1958; Montgomery, 1997). In some situations, a large number of factors that might influence the results of an experiment can be studied efficiently using more advanced factorial designs. For example, in screening potential drugs, it may be desirable to choose a suitable combination of variables (e.g., presence/absence of the test compound; the sex, strain, age, and diet of the animals; time after treatment; and method of measuring the endpoint). This type of design can also be used to optimize experiments that are used repeatedly with only minor changes in the treatments, such as in drug development, when many different compounds are tested using the same animal model (Shaw et al., 2002).

6. **Dependent Variable(s):**

When changes in one variable depend upon the changes in other variable or variables, it is known as a dependent or endogenous variable. In animal related protocols, dependent variables are those one or a few outcomes of interest that we get after experimentation, which are typically mentioned in the experimental hypotheses. For example, the hypothesis might be that the experimental treatments do not affect body weight in rats. Generally, there should be very few outcomes of primary interest. However, in some experimental units like in case of toxicity experiments many dependent variables are there, any of which may be altered by a toxic chemical. Thus, treatment is independent variable while body weight is dependent, as the treatment can affect it. Therefore, researcher should be aware of all dependent variables while designing the study.

7. **Independent Variables or Treatments:**

Positive or negative control groups. In case of animal studies, many exogenous factors can affect the ultimate outcome of the study and can affect the hypothesis by altering the dependent variable. Therefore, the external variables that cause the changes in the dependent variable are known as the independent or explanatory or exogenous variables. For example, if demand depends upon price, then demand is a dependent variable, while price is the independent variable. For example in animal studies the experiments usually involve the deliberate alteration of some treatment factor such as the dose level of a drug. The treatments may include one or more "controls." Negative controls may be untreated animals or those treated with a placebo without an active ingredient. The latter is normally more appropriate, although it may be desirable to study both the effect of the active agent and the vehicle, in which case both types of control will be needed. Thus, the treatment with vehicle control without active ingredient is important independent variable as sometime the vehicle in which the active druq is administered or dissolved could per se affect the result (dependent variable). Therefore, effect of exogenous factors on the dependent variables can lead to false positive or negative results. Similarly, to avoid the interference of the independent variable on the research outcome, even in surgical studies may involve sham-operated controls, which are treated in the same way as the tested animals but without the final surgical treatment. Positive controls are sometimes used to ensure that the experimental protocols were actually capable of detecting an effect. All these points emphasizes on the importance of maintaining all the positive or negative treatment, and surgical controls to negate the influence of independent or explanatory or exogenous variables in research design.

8. **Uncontrolled (Random) Variables:**

Environmental factors: and In addition to the treatment variables, there may be a number of random variables that are uncontrollable yet may need to be taken into account in designing an experiment analyzing the results. For example, circadian rhythms may cause behavior measured in the morning to be different from that measured in the afternoon. Similarly, the experimental material may have some natural structure (e.g. members of a litter of mice may be more similar than animals of different litters). Measurements made by different people or different times may be slightly different, and reagents may deteriorate over a period of time. If these effects are likely to be large in relation to the outcomes being investigated, it will be necessary to account for them at the design stage (e.g. using a randomized block, Latin square, or other appropriate design) or at the time of the statistical analysis (e.g. using covariance analysis).

9. **Ethical approval:**

In the pharmacological screening, after proper designing the research protocols researcher should seek a ethical approval from the competent authority for conducting the protocols on animals. In India, the ethical approval is taken by submitting a form application to the internal/institutional ethics committee (IAEC) which is constituted by CPCSEA, New Delhi in the researcher institute. The IAEC will scrutinize the research application submitted by researcher for reinforcing the ethics and guideline laid down by the CPCSEA, New Delhi and if required will give suggestion or alter on number/type/surgical procedure/ protocol to be conducted on animals. The researcher can start experimental work on the submitted research project only after IAEC ethical clearance.

STEP FOUR:

Preliminary data Collection:

This step start after researcher have posed the research question, and planned research well, for collecting the data. A methodical approach is recommended considering all the points discussed in step three by continually measurement of the performance against the work plan, and making adjustments where necessary. Moreover, on encountering the problems during the course of study, they need to be solved, or alternative courses of action planned. Thus, considering all these points the preliminary data should be collected and if needed further new protocols are designed to address the question rose from the preliminary data interpretation for proving the hypothesis. At this point, the research data is applied with statistic for checking the level of significance to check whether the treatment have affected the parameters noted with respective to the control groups.

STEP FIVE AND SIX:

Statement and conclusion: Returning to research question: After collecting and interpretation of research results, researcher should to return to the research question/s. This is the most important way of getting research answer and will be a good guide in helping the researcher to arrange results. Generally, two type of research data are collected quantitative and qualitative data, which needs to be analysed to put forward the statement and conclusion of the research topic/studies conducted. Following points are taken in consideration while analysing the data: Analysing quantitative data

1. Save your document/s with your original data in a separate folder on your computer.

If you are working with paper documents, consider whether it is necessary to make photocopies of your results so that the originals can be filed away securely. Work off the copies, not the master documents. This is a good thing to do with any of your research results. 2. Tabulate the information.

For instance, add up the number of responses you received for your survey and categorize them in an appropriate way by referring to your research question (e.g. the number of yes and no answers for each question; male and female responses or racial categories). Work out what your tabulated answers are saying. For instance, convert some of your sums into percentages. Say 30% of the respondents said X, while 25% said Y. These are easier to internalize for those reading your report.

4. Try to be creative.

Once you have tabulated and calculated your results, there may be some interesting and unexpected interpretations of the data that can be made.

5. Double-check all your calculations.

Analysing qualitative data:

Read through all the data, making notes of associations or ideas that occur to you. Organize the data into similar categories (e.g. responses to particular questions; or categories of informants, such as government representatives, members of the community or newspaper editors). Attempt to identify patterns or associations and causal relationships in the themes (e.g. responses from people in the same geographic area, from the same income group or the media preferences of people who do not have electricity at home). Be creative and analytical. If you have done quantitative research at the same time, try to match some of your qualitative results to your quantitative results. Where are the links? How do the results 'speak to' or explain each other? What conclusions can be reached that are not obvious at first glance? Conclusions: Be methodical. Think step by step, and explain your assumptions if you have to. Remember, your research results might not tell you everything you want to know but do not be afraid to say what they do tell you. Be careful with your words, and be specific.

STUDENT-T-TEST

The Student's *t*-test is one of the most important statistical tests used in research to determine whether there is a significant difference between the means of two groups. It is widely applied in fields such as pharmaceutical research, biomedical sciences, psychology, and clinical studies. The test was developed by the statistician William Sealy Gosset, who published his work under the pseudonym "Student."

The *t*-test is particularly useful when the sample size is small (usually less than 30) and the population standard deviation is unknown. In such situations, the test uses the sample data to estimate the population parameters and determine whether the difference between groups is statistically meaningful or occurred by chance.

. **Definition of Student's *t*-Test**

The Student's *t*-test is a parametric statistical test used to compare the means of one or two samples to determine whether they are significantly different from each other.

In simple terms, the *t*-test answers the question:

Is the difference between the two sample means large enough to conclude that the populations from which they were drawn are different?

If the calculated *t*-value exceeds the critical value obtained from the *t*-distribution table, the null hypothesis is rejected, indicating that a significant difference exists between the groups.

2. **Objectives of the *t*-Test**

The major objectives of using Student's *t*-test include:

1. To compare the mean of a sample with the population mean.
2. To compare the means of two independent groups.
3. To compare measurements of the same group at different times.
4. To determine whether the difference between two groups is statistically significant.

In pharmaceutical and biomedical research, the *t*-test is often used to compare drug-treated groups with control groups to evaluate the effectiveness of a drug.

3. **Assumptions of Student's *t*-Test**

Before applying the *t*-test, certain assumptions must be satisfied:

1. Normal Distribution
   The data should follow a normal (Gaussian) distribution.
2. Independence of Observations
   The observations should be independent of each other.
3. Small Sample Size
   The test is commonly used when the sample size is small ($n < 30$).
4. Continuous Data
   The data should be measured on an interval or ratio scale.
5. Equal Variance (for independent t-test)
   The variance of the two groups should be approximately equal.

If these assumptions are violated, non-parametric tests such as the Mann–Whitney U test may be used instead.

4. **Types of Student's *t*-Tests**

There are three main types of Student's *t*-tests:

1. One-Sample *t*-Test

The one-sample *t*-test is used to compare the mean of a single sample with a known population mean.

Example:
If a pharmaceutical company claims that a drug reduces blood pressure by 10 mmHg, a researcher may test a sample of patients to determine whether the average reduction is significantly different from 10 mmHg.

Formula:

Formula:

$$t = \frac{\bar{X} - \mu}{s/\sqrt{n}}$$

Where:

- $\bar{X}$ = sample mean
- μ = population mean
- s = sample standard deviation
- n = sample size

2. **Independent (Unpaired) *t*-Test**

The independent *t*-test is used when two separate groups are compared.

Example:
Comparing the effect of a new antihypertensive drug in a treated group versus a control group receiving placebo.

In this test, the participants in one group are different from those in the other group, and there is no relationship between the observations.

Formula:

Formula:

$$t = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}}$$

Where:

- $\bar{X}_1, \bar{X}_2$ = means of two groups
- s_1, s_2 = standard deviations
- n_1, n_2 = sample sizes

3. **Paired (Dependent)** ***t*****-Test**

The paired *t*-test is used when two sets of observations are related or paired.

This usually occurs when measurements are taken on the same subjects before and after treatment.

Example:

- Blood glucose levels of patients before and after drug treatment
- Body weight before and after diet therapy

In this test, the difference between each pair of observations is calculated first.

Formula:

t=d¯sd/nt = \frac{\bar{d}}{s_d/\sqrt{n}}t=sd/nd¯

Where:

- d¯\bar{d}d¯ = mean difference between paired observations
- sds_dsd = standard deviation of the differences
- nnn = number of pairs

5. **Steps in Performing Student's *t*-Test**

The following steps are usually followed when performing a *t*-test:

Step 1: Formulate Hypotheses

Two hypotheses are formulated:

- Null Hypothesis (H_0): There is no significant difference between the means.
- Alternative Hypothesis (H_1): There is a significant difference between the means.

Step 2: Choose Significance Level

The significance level (α) is usually set at 0.05 (5%) or 0.01 (1%).

Step 3: Calculate the *t*-Value

The *t*-value is calculated using the appropriate formula depending on the type of *t*-test.

Step 4: Determine Degrees of Freedom (df)

For example:

- One sample: df=n−1df = n - 1df=n−1
- Independent samples: df=n1+n2−2df = n_1 + n_2 - 2df=n1+n2−2

Step 5: Compare with Critical Value

The calculated *t*-value is compared with the critical value from the *t*-distribution table.

Step 6: Draw Conclusion

- If t calculated > t table → Reject null hypothesis
- If t calculated ≤ t table → Accept null hypothesis

6. Applications of Student's *t*-Test

Student's *t*-test has many applications in scientific research:

1. Pharmaceutical Research

It is used to compare the efficacy of drugs in experimental and control groups.

2. Clinical Trials

The test helps determine whether a new treatment produces significant improvement in patients.

3. Toxicology Studies

Researchers compare biochemical parameters before and after exposure to toxic substances.

4. Pharmacology Experiments

The test is used to analyze animal model experiments to determine drug activity.

5. Quality Control in Industry

The *t*-test can be used to compare batch quality or production processes.

7. **Advantages of Student's *t*-Test**

1. Simple and easy to calculate
2. Suitable for small sample sizes
3. Widely applicable in biomedical research
4. Provides reliable statistical inference
5. Useful when population variance is unknown

8. **Limitations of Student's *t*-Test**

Despite its advantages, the *t*-test has certain limitations:

1. It assumes normal distribution of data.
2. It is sensitive to outliers.
3. It cannot compare more than two groups simultaneously.
   In such cases, Analysis of Variance (ANOVA) is used.
4. The results may be inaccurate if sample size is extremely small or variances are unequal.

ANOVA

Analysis of Variance (ANOVA) is a statistical method used to **compare the means of three or more groups simultaneously** to determine whether there is a statistically significant difference among them. Instead of performing multiple *t*-tests (which increases the risk of error), ANOVA analyzes the **variance within groups and between groups** to determine if at least one group mean differs from the others.

The method was developed by the statistician **Ronald A. Fisher**, who introduced it as part of modern experimental design and statistical analysis.

ANOVA is widely used in **pharmaceutical research, clinical trials, biology, agriculture, and social sciences** to analyze experimental data involving multiple treatments or groups.

Principle of ANOVA

The basic principle of ANOVA is based on the **comparison of two types of variances**:

1. **Variance between groups (Treatment variance)**
   This measures how much the group means differ from the overall mean.
2. **Variance within groups (Error variance)**
   This measures the variability of observations within each group.

If the **between-group variance is significantly greater than the within-group variance**, it indicates that the treatment or factor has a significant effect.

ANOVA calculates an **F-ratio (F-statistic)**, which is the ratio of these two variances:

F=Variance between groups/ Variance within groups

- If **F value is large**, it suggests significant differences between group means.
- If **F value is small**, the differences between group means are likely due to random variation.

Objectives of ANOVA

The main objectives of ANOVA are:

1. To determine whether **three or more group means differ significantly**.
2. To analyze the **effect of different treatments or factors** on a response variable.
3. To **separate total variation** in the data into different components.
4. To **avoid multiple t-tests**, which increase the probability of error.

Assumptions of ANOVA

Before applying ANOVA, the following assumptions must be satisfied:

1. **Normal distribution**
   The data in each group should follow a normal distribution.
2. **Independence of observations**
   Observations should be independent of each other.
3. **Homogeneity of variance**
   Variances of all groups should be approximately equal.
4. **Random sampling**
   Samples should be randomly selected from the population.

Violation of these assumptions may lead to incorrect results.

Types of ANOVA

There are mainly two types of ANOVA used in research.

1. One-Way ANOVA

One-way ANOVA is used when **one independent variable (factor)** is studied with **three or more groups**.

Example:
Comparing the **effect of three different drugs on blood pressure**.

Groups:

- Drug A
- Drug B
- Drug C

The factor is **type of drug**, and the response variable is **blood pressure reduction**.

2. Two-Way ANOVA

Two-way ANOVA is used when **two independent variables (factors)** affect the dependent variable.

Example:

Studying the effect of:

- **Drug type**
- **Dosage level**

on blood pressure reduction.

Two-way ANOVA can analyze:

- Individual effect of each factor
- Interaction effect between factors

Steps in Performing ANOVA

The following steps are followed to perform ANOVA.

Step 1: State the Hypotheses

Two hypotheses are formulated.

Null Hypothesis (H_0)

All group means are equal.

μ1=μ2=μ3=...=μk\mu_1 = \mu_2 = \mu_3 = ... = \mu_kμ1=μ2=μ3=...=μk

Alternative Hypothesis (H_1)

At least **one group mean is different**.

Step 2: Select Significance Level

The significance level (α) is usually chosen as:

- **0.05 (5%)**
- **0.01 (1%)**

This represents the probability of rejecting the null hypothesis when it is actually true.

Step 3: Calculate Group Means and Overall Mean

First, calculate:

- Mean of each group

- Overall mean (grand mean)

The **grand mean** is the average of all observations.

Step 4: Calculate Sum of Squares

ANOVA divides total variation into components using **Sum of Squares (SS)**.

1. Total Sum of Squares (SST)

Represents the total variability in the data.

SST=∑(X−X¯)2SST = \sum (X - \bar{X})^2SST=∑(X−X¯)2

Where:

- XXX = individual observation
- X¯\bar{X}X¯ = grand mean

2. Sum of Squares Between Groups (SSB)

Represents variation due to differences between group means.

SSB=∑n(Xi¯−X¯)2SSB = \sum n(\bar{X_i} - \bar{X})^2SSB=∑n(Xi¯−X¯)2

Where:

- Xi¯\bar{X_i}Xi¯ = group mean
- nnn = number of observations in each group

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3. Sum of Squares Within Groups (SSW)

Represents variation within each group.

SSW=∑(Xij−Xi¯)2SSW = \sum (X_{ij} - \bar{X_i})^2SSW=∑(Xij−Xi¯)2

Step 5: Calculate Degrees of Freedom

Degrees of freedom are calculated for each component.

Source of Variation	Formula
Between Groups	$k - 1$
Within Groups	$N - k$
Total	$N - 1$

Where:

- **k** = number of groups
- **N** = total number of observations

Step 6: Calculate Mean Squares

Mean squares are obtained by dividing the sum of squares by degrees of freedom.

$$MS = \frac{SS}{df}$$

Mean Square Between Groups (MSB)

$$MSB = \frac{SSB}{k-1}$$

Mean Square Within Groups (MSW)

$$MSW = \frac{SSW}{N-k}$$

Step 7: Calculate F-Statistic

The **F-ratio** is calculated as:

F=MSBMSWF = \frac{MSB}{MSW}F=MSWMSB

This value indicates whether group means differ significantly.

Step 8: Compare with F-Table Value

The calculated **F value** is compared with the **critical value from the F-distribution table**.

Decision rule:

- If **F calculated > F table** → Reject null hypothesis
- If **F calculated ≤ F table** → Accept null hypothesis

Step 9: Draw Conclusion

If the null hypothesis is rejected, it indicates that **at least one group mean is significantly different**.

However, ANOVA does not identify **which specific groups differ**. For this purpose, **post-hoc tests** such as **Tukey's Honestly Significant Difference Test** or **Bonferroni correction** are used.

ANOVA Table (Summary Table)

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F-ratio
Between Groups	SSB	k−1	MSB	MSB/MSW
Within Groups	SSW	N−k	MSW	
Total	SST	N−1		

This table summarizes the entire ANOVA calculation.

Applications of ANOVA

ANOVA is widely used in many scientific fields.

Pharmaceutical Research

To compare the **effectiveness of different drugs or doses**.

Clinical Trials

To analyze **treatment effects among multiple patient groups**.

Agriculture

To compare **crop yields using different fertilizers**.

Biology

To study **effects of environmental factors on organisms**.

Psychology and Social Sciences

To compare **behavioral responses between groups**.

Advantages of ANOVA

1. Can compare **more than two groups simultaneously**.
2. Reduces the **risk of Type I error** compared to multiple t-tests.
3. Provides a **systematic analysis of variation**.
4. Widely applicable in **experimental research**.

Limitations of ANOVA

1. It only tells **that a difference exists**, not **which groups differ**.
2. Requires **normal distribution of data**.
3. Sensitive to **unequal variances**.
4. Requires additional **post-hoc tests** for detailed comparison.

Graphical Representation of Data

Graphical representation of data refers to the presentation of numerical information in a **visual form using graphs, charts, and diagrams**. It helps in **simplifying complex data**, making it easier to understand, analyze, and interpret. Graphical representation is widely used in **statistics, pharmaceutical research, clinical studies, economics, and social sciences** to display patterns, trends, and relationships between variables.

Instead of analyzing large tables of numbers, graphs and diagrams allow researchers to **quickly identify trends, comparisons, and variations**. Visual representation also improves communication of data in **research reports, presentations, and publications**.

Objectives of Graphical Representation of Data

The main objectives of graphical representation are:

1. **Simplification of Data**
   Complex numerical data can be presented in a simple and understandable visual form.
2. **Quick Comparison**
   Graphs help compare values of different categories easily.
3. **Identification of Trends**
   Changes over time or patterns can be easily observed.
4. **Better Interpretation**
   Researchers can interpret relationships between variables more clearly.
5. **Effective Presentation**
   Graphs and diagrams make research reports more attractive and informative.

Types of Graphical Representation

Graphical representation of data is mainly divided into two categories:

1. **Diagrams**
2. **Graphs**

Both methods visually represent data but differ in their purpose and structure.

1. Diagrammatic Representation of Data

Diagrams are **simple visual presentations of data** using shapes, bars, and pictures. They are easy to understand even for people without statistical knowledge.

Types of Diagrams

1. Bar Diagram

A **bar diagram** represents data using **rectangular bars of equal width** where the length of each bar corresponds to the value of the variable.

Types of Bar Diagrams:

- **Simple bar diagram**
- **Multiple bar diagram**
- **Component bar diagram**
- **Percentage bar diagram**

Example:
A bar diagram can show the **number of patients responding to different drugs** in a pharmacological study.

Characteristics

- Bars are of equal width
- Bars are separated by equal space
- Height of bar represents the value

Advantages

- Easy to construct
- Easy to interpret
- Useful for comparison

2. Pie Diagram (Pie Chart)

A **pie chart** is a circular diagram that represents data as **proportions of a whole**. The circle is divided into sectors, and each sector represents a category.

Each sector angle is calculated using:

Angle=ValueTotal×360∘\text{Angle} = \frac{\text{Value}}{\text{Total}} \times 360^\circAngle=TotalValue×360∘

Example:
A pie chart can represent the **percentage distribution of different diseases in a hospital**.

Advantages

- Clearly shows proportions
- Attractive and easy to understand

Limitations

- Not suitable for large datasets
- Difficult to compare many categories

3. Pictogram

A **pictogram** uses pictures or symbols to represent data.

Example:
One symbol may represent **10 patients** in a hospital survey.

Advantages

- Visually appealing
- Easy to understand for general audiences

Disadvantages

- Not very precise
- Difficult to represent large datasets

4. Histogram

A **histogram** is a graphical representation of **continuous frequency distribution**.

It consists of **rectangles where the area of each rectangle corresponds to the frequency**.

Characteristics:

- No gaps between bars
- Used for continuous data
- Shows distribution of variables

Example:
Histogram showing **distribution of blood pressure levels in patients**.

5. Frequency Polygon

A **frequency polygon** is obtained by **joining the midpoints of the tops of histogram bars** using straight lines.

It is often used to **compare multiple frequency distributions**.

Example:
Comparing **drug effectiveness in two different treatment groups**.

2. Graphical Representation Using Graphs

Graphs represent the **relationship between two variables**, usually plotted on **two axes**.

- **X-axis (horizontal axis)** – independent variable
- **Y-axis (vertical axis)** – dependent variable

1. Line Graph

A **line graph** shows the relationship between variables over time by connecting points with lines.

Example:
A line graph can represent **change in blood glucose levels over time after drug administration**.

Applications:

- Time series data
- Clinical trial monitoring
- Growth patterns

Advantages:

- Shows trends clearly
- Easy to interpret changes over time

2. Scatter Diagram (Scatter Plot)

A **scatter diagram** shows the relationship between two variables using individual points plotted on a graph.

Example:
Relationship between **drug dose and therapeutic response**.

Scatter plots help identify **correlation**:

- Positive correlation
- Negative correlation
- No correlation

Statistical methods like **Pearson correlation coefficient** are often used to measure the strength of this relationship.

3. Ogive Curve

An **ogive** is a **cumulative frequency curve**.

Types:

- **Less than ogive**
- **More than ogive**

Uses:

- Determining **median**
- Determining **quartiles**
- Analyzing cumulative data

Steps for Constructing Graphs

The following steps are followed when constructing graphs:

1. **Collect and organize data**
   Arrange data in tabular or frequency distribution form.
2. **Select appropriate graph type**
   Choose bar graph, histogram, line graph, etc., depending on the data.
3. **Draw axes**
   - Horizontal axis (X-axis)
   - Vertical axis (Y-axis)
4. **Select suitable scale**
   Choose scale so that data fits properly on the graph.
5. **Plot data points**
   Mark values accurately on the graph.
6. **Label axes and title**
   Provide a clear title and label units.
7. **Interpret the graph**
   Analyze patterns, trends, or relationships.

Advantages of Graphical Representation

1. **Simplifies complex data**
2. **Facilitates comparison between groups**
3. **Helps identify trends and patterns**
4. **Improves presentation of research findings**
5. **Useful for quick interpretation**

Limitations of Graphical Representation

1. Graphs may **oversimplify complex data**.
2. They **do not provide exact numerical values**.
3. Poorly designed graphs may **mislead interpretation**.
4. Requires proper scaling and labeling for accuracy.

Applications in Pharmaceutical and Biomedical Research

Graphical representation is widely used in:

- **Drug efficacy studies**
- **Clinical trial results**
- **Pharmacokinetic studies**
- **Toxicity studies**
- **Biostatistical analysis**

For example, dose–response relationships in pharmacology are often represented using graphs such as the **Dose–response relationship** curve.

IMPORTANT QUESTIONS

Very Short Questions (2 marks each)

1. What is a research hypothesis?
2. Define "review of literature."

3. What is the main purpose of selecting a research topic?
4. What does ANOVA stand for?
5. Explain the term "study design."
6. What is the Student's t-test used for in pre-clinical research?
7. Name one method of graphical representation of data.
8. Define "one-way ANOVA."
9. What is the significance of data interpretation in research?
10. Mention one advantage of using graphical representation in data analysis.

Short Questions (5 marks each)

1. Describe the process of selecting a research topic.
2. Explain the importance of the review of literature in research.
3. Discuss the components of a research hypothesis.
4. Illustrate the basic steps involved in designing a study.
5. Explain how the Student's t-test is used to analyze pre-clinical data.
6. Describe the procedure of one-way ANOVA and its application.
7. Summarize the different types of graphical representation of data.
8. Discuss the significance of interpreting pre-clinical data accurately.
9. Explain how one-way ANOVA differs from the Student's t-test.
10. Describe the role of graphical representation in summarizing research findings.

Long Questions (10 marks each)

1. Analyze the importance of a well-defined research hypothesis and its impact on study design.
2. Evaluate the process of conducting a thorough review of literature and its influence on research outcomes.
3. Discuss the different types of study designs and their applications in pre-clinical research.

4. Critically assess the use of the Student's t-test in pre-clinical data analysis, highlighting its strengths and limitations.
5. Describe the detailed procedure of conducting a one-way ANOVA and interpreting its results.
6. Compare and contrast one-way ANOVA and the Student's t-test in the context of pre-clinical research.
7. Discuss the various methods of graphical representation of data and their effectiveness in conveying research findings.
8. Evaluate the challenges and best practices in interpreting pre-clinical data to ensure accurate conclusions.
9. Analyze the role of bio-statistics in enhancing the reliability and validity of pre-clinical research.
10. Discuss the integration of research methodology and bio-statistics in the development of a comprehensive pre-clinical study.