UNIT-V

Research methodology and Bio-statistics

Points to be covered in this topic

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

SELECTION OF RESEARCH TOPIC, REVIEW OF LITERATURE, RESEARCH HYPOTHESIS AND STUDY DESIGN

INTRODUCTION TO RESEARCH

- 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"

TYPES OF RESEARCH

Types of Research	Description
Descriptive versus Analytical	• **Descriptive research** includes surveys/scientific reviews and fact-finding enquiries of different kinds. The major purpose of descriptive research is compiling of the state of scientific or general affairs, as it exists at present. • **Analytical research**, the researcher uses scientific facts or information already available, and analyze these to make a critical evaluation of the scientific material.
Applied versus Fundamental	• **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. • **Fundamental research** is mainly concerned with generalisations and with the formulation of a theory i.e. as said "Gathering knowledge for knowledge's sake is termed as 'pure' or 'basic' research."

Quantitative versus Qualitative	• **Quantitative research** is based on the measurement of quantity or amount. • **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.
Conceptual versus Empirical	• Research related to some abstract ideas 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". • **Empirical research** is data-based research or experimental type of research in which, researcher comes to conclusion that can be verified by observation or experiment.

RESEARCH PROCESS

❖ **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

The first stage in the research process is to select and properly define the research question.

- **What? i.e. What do I want to know?**
- **How? - How do I find out what I want to know?**
- **And why? - Finding the right answers to the research question.**

STEP TWO: Review of literature and Research hypothesis

Review of literature:

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.
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.

STEP THREE: Research Methodologies i.e. to find out how the study should be conducted?

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.
- Ethical clearance for animal/human study.
- Total cost of the research project.

Types of research design

❑ Exploratory Research Design:

The **exploratory research design** is also known as **formulative research design** as it formulates a research problem for an **in-depth or more precise investigation, or for developing a working hypothesis** from an operational aspect.

❑ 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.**

❑ Hypothesis testing research design:

Hypothesis-Testing Research Designs are those in which the **researcher tests the 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.**

✓ **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 Variables
7. Independent Variables or Treatments
8. Uncontrolled (Random) Variables
9. Ethical approval

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.

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 questions. This is the most important way of getting research answer and will be a good guide in helping the researcher to arrange results.

PRE-CLINICAL DATA ANALYSIS AND INTERPRETATION USING STUDENTS 't' TEST AND ONE-WAY ANOVA

❖ Quantitative Data:

Quantitative data are often summarized in terms of the mean, "n" (the number of subjects), standard error mean (SEM) and the standard deviation (SD) as a measure of variation.

Quantitative data can be analyzed using following approach:

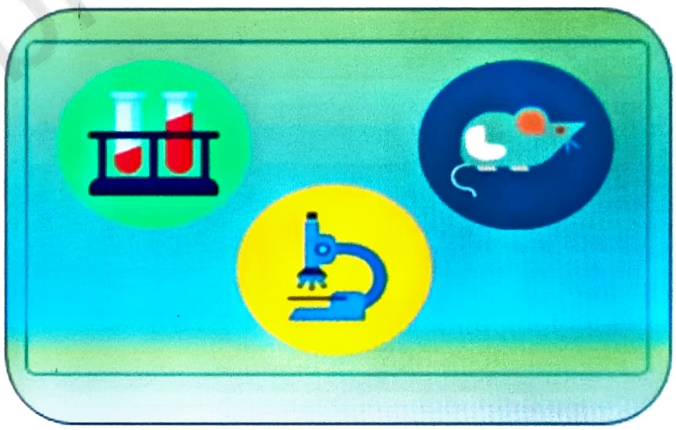
➢ Parametric methods:

Parametric tests are usually more versatile and powerful and so are preferred for analysis of the data statistically.

1. The t-test used for one or two groups.
2. The analysis of variance (ANOVA) for more than two or several groups.

➢ Nonparametric methods:

1. Mann-Whitney test.



t-Test and ANOVA:

t-Test:

- This test can be directly applied to a data using various statistical software.
- The t-test is used to test differences in mean between two groups.
- The t-test is used when the dependent variable is a continuous interval/ratio scale variable and the independent variable is a two-level categorical variable.
- The t-test can be used even if sample sizes are very small, as long as the variables within each group are normally distributed and the variation of scores within the two groups is equal.

Types of t-test: Depends on the type of data to be analyzed:

Independent samples t-test	Paired samples t-test
The t-test can be used to analyze the data wherein there are two independent groups	When the sample is paired or dependent. Dependent samples are two groups matched on some variable or the same group being tested twice
An example of an independent samples t-test is evaluating differences in test scores between a group of patients who were given a treatment intervention and a control group who received a placebo	An example of a paired samples t-test is computing differences in test scores on the same sample of patients using a pre-test-post-test design

✓ Interpretation of t-test:

In t-test, the test statistic is used to generate p (probability) values, which has a student's t distribution with n-1 degrees of freedom. In other words, in t-test if the **p-value is $p < 0.05$ i.e. there exists a ~5% chance of getting the observed differences between the two group's readings.**

Effect size:

When only two groups are to be compared, the effect size is the difference in means or proportions that the investigator wants the experiment to be able to detect. Effect size is a population effect and its indices are independent of sample size.

✓ Cohen's d:

The effect size (d) is the difference between the two population means, divided by the estimated population standard deviation.

✓ eta squared:

The formula for eta squared = t_2/t_2 + (N12 + N2-2)

✓ Confidence interval (CI):

A confidence interval (CI) is formulated in order to ascertain how precise is the estimate of effects. The CI is constructed around a sample mean or another statistic to establish a range of values for the unknown estimated population parameter, as well as the probability of being right.

Information to be furnished in results after the t-test analysis:

When a researcher reports the results from an independent or paired-samples t-test, he or she needs to include the following information:

1. Verification of parametric assumptions.
2. Dependent variable scores.
3. Independent variable, levels.
4. Statistical data: Significance, t-scores, probability, group means (M), group SD/standard error mean (SEM), mean differences, CI, and effect size.

ANOVA

- ANOVA is a statistical analysis used to compare means between three or more groups.
- In ANOVA, though it is called as analysis of variance but it is concerned with differences between means of groups, not differences between variances.

Types of ANOVA:

1. One-way ANOVA:

In which there is only one-factor. Example: effect of various doses of caffeine on locomotor activity of mice. In this case, factors are the independent variables, each of which must be measured on a categorical scale.

There are two different types of one-way ANOVA:

(a) Between groups ANOVA: Comparing two or more different groups; independent design.

(b) Repeated measures ANOVA: One group of subjects exposed to two or more conditions; within-subjects design.

2. Two-way ANOVA:

In which there is only two-factor. Example: effect of saline or caffeine treatment on locomotor activity of mice on day 1, day 3, day 5, day 7, day 9, day 11, day 13.

Following information is generated using ANOVA and is furnished in results after the ANOVA analysis :

When a researcher reports the results from an ANOVA test, he or she needs to include the following information:

1. Verification of parametric assumptions.

2. Dependent variable scores.

3. Independent variable levels.

4. Statistical data:

(a) Significance

(b) F-ratio scores: F-ratio is the ratio of the mean of the squares between to the mean of the squares within:

F = MSb/MSW (each of the means = SS/df).

Like the t-test, F depends on degrees of freedom to determine probabilities and critical values.

(c) Probability (p).

(d) Group M.

(e) Group SD/SEM.

(f) Mean differences.

(g) Confidence intervals.

(h) Effect size.

(i) P-value for between group difference using post hoc test.

Following are some of the nonparametric tests commonly employed:

1. The Wilcoxon rank sum test and the Mann-Whitney test: Used to compare two groups and constitute a nonparametric equivalent of the two-sample t-test.

2. The Kruskal-Wallis test: It compares several groups and is the nonparametric equivalent of the one-way ANOVA.

3. The Friedman test: It is the nonparametric equivalent of the randomized block ANOVA for more than two treatment groups.

GRAPHICAL REPRESENTATION OF DATA

INTRODUCTION

- Graphical representation is one of the easiest ways to analyse a numerical data.
- Graph is a sort of chart through which statistical data are represented in the form of lines or curves drawn across the coordinated points plotted on its surface such that it becomes easy to study the cause and effect relationship between two variables.

TYPE OF DATA: Choice of Graph is Dependent on the Type of Data

There are several different variables depending on which, data can be classified as follows:

Variable:

- A variable is an item of data.
- Examples of variables include quantities such as: gender, treatments, time, day, test scores, and weight.
- The values of these quantities vary from one observation to another.

Types/Classification of Variables

1. Qualitative Data: This data describes the quality of something in a non-numerical format. Counts can be applied to qualitative data, but you cannot order or measure this type of variable. Examples are gender, marital status, geographical region of an organization, job title etc.

Measurement Scale:

- **Nominal:** classifies with **no ranking** (e.g. Male, female type).
- **Ordinal:** classifies with **ranking** (e.g. Grades, activity count).

Graphical representation or Analysis of qualitative data:

This can be done using following ways:

- Frequency tables, Contingency tables (for two variables).
- Modes Most frequently occurring.

2. Quantitative Data:

Quantitative or numerical data **arise when the observations are frequencies or measurements.**

(a) Discrete Data: The data are said to be discrete if the measurements are integers **(e.g. number of animals, number of correct answers on a test etc.)**

(b) Continuous Data: The data are said to be continuous if the **measurements can take on any value,** usually within some range **(e.g. weight of animal).** Age and weight are continuous quantitative variables.

Graphical representation or Analysis of qualitative data:

This can be done using any of the following approaches:

- **Tables:** Create **groups or categories and generate frequency** tables.
- **Graphs: Histograms, Stem-and-Leaf plots, Dot Plots, Box plots,** and XY Scatter Plots.

Measurement Scale:

- **Interval:** ordered and difference between **variables is meaningful.**
- **Ratio:** ordered and difference between variables is meaningful, **true 0 in measuring.**

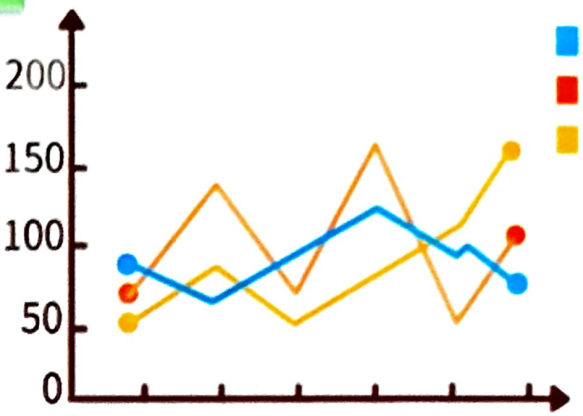
BASIC PRINCIPLES OF GRAPHICAL REPRESENTATION

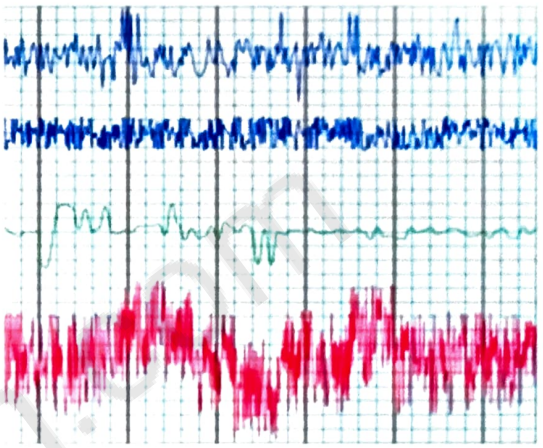
All types of graphical representation of data are **governed by some algebraic principles,** in which there are two lines called **coordinate axes** i.e. a vertical known as **Y-axis** and a horizontal called **X-axis.**

Types of graphical representations

1. Line diagrams/graph:

Line diagrams are drawn by **plotting the values of two continuous variables.** Line diagrams may be presented in the form of **continuous lines** or **segmented lines** depending on the phenomenon under study.





2. Polygraph: Is a line graph in which **two or more than two variables are shown on a same diagram by different lines.** It helps in comparing the data.

3. Bar Graphs: The bar graphs are also called as **columnar diagrams.** The bar diagrams are **drawn through columns of equal width.**

- **Following rules should be taken in consideration while constructing a bar diagram:**

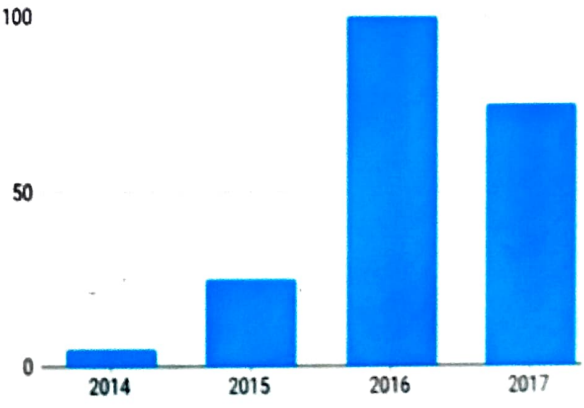
(a) The bars or columns should have **equal width.**

(b) An **equal intervals/distance should be present** between all the bars.

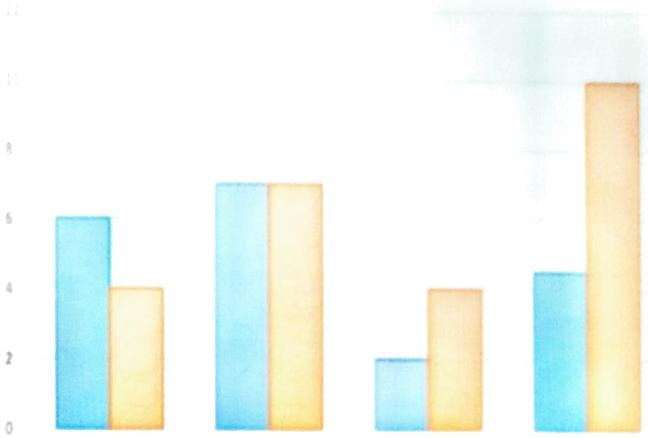
(c) All **bars should be made attractive and distinct** by applying shades with colours or patterns.

- **Bar graphs are sub-classified into following types to represent different data sets:**

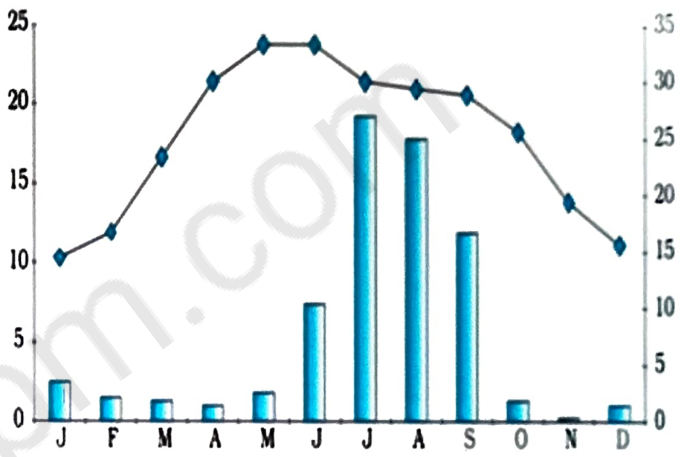
(a) The simple bar diagram: Immediate comparison of a data can be easily made by constructing this type of bar graph. In this type the **data set is generally arranged in an ascending or descending order.**



(b) Compound bar diagram: A compound bar diagram is generally a choice when different components are needed to be grouped in one set of variable or different variables of one component are put together.



(b) Polybar diagram: In this type of bar graph both the line and bar graphs are combined to depict the data related to some of the closely associated characteristics such as the data of mean of number of marbles buried and effect on locomotor count after drug administration.



4. The Pie Diagram:

Using the pie diagram the total value of the given attribute can be drawn using a circle, in which the circle is divided into corresponding degrees of angle to represent the sub- sets of the data. Therefore, it is also called as divided circle diagram.