THEORY FILE

AIM:- Biostatistical method in experimental pharmacology – ANOVA (Analysis of Variance)

INTRODUCTION

- Biostatistical methods in experimental pharmacology involve the application of statistical tools to design experiments, analyse data, and draw valid conclusions about drug effects. These methods help ensure reliability, minimize bias, and determine significance in drug testing.
- For analysis of data, we use T-test, ANOVA (Analysis of Variance), Regression

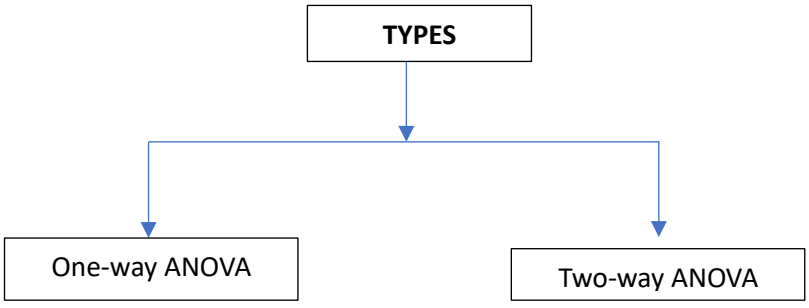
ANOVA (Analysis of Variance)

ANOVA (Analysis of Variance) is a statistical method used to compare means of three or more groups to determine if there are significant differences between them. It basically observes difference obtained are either by-chance or it's real.

Purpose: In experimental pharmacology, ANOVA helps analyse data from drug trials and experiments to assess the effect of different treatments or dosages. ANOVA separates the total variability observed in the data into components attributable to different sources (e.g., treatment effect and random error).

Why Use ANOVA Over Multiple t-tests?

ANOVA controls the Type I error rate that increases when multiple t-tests are used for comparisons among several groups.



ASSUMPTIONS:-

- Each Sample taken are normally distributed.
- Sample drawn are independent and random of another sample.
- Variance of data in different group should be same.

FORMULA:-

ANOVA= Variance Between / Variance Within

Key Terms:

- **F-ratio**: The ratio of variance between groups to variance within groups.
- **P-value**: Indicates the probability that observed differences occurred by chance.

INTERPRETATION

The test output includes an F-statistic and p-value. A high F-statistic value and low p-value ($p < 0.05$) lead to rejection of null hypothesis.

Null Hypothesis- The means for all groups are the same

Alternate Hypothesis:- The means are different for at least one pair of groups.

EXAMPLE: -

To compare the analgesic effect of three different drugs (A, B, and C) by measuring tail-flick latency in rats.

Experimental Design

- **Subjects**: 15 rats (5 per group).
- **Groups**:
    - Group 1: Drug A
    - Group 2: Drug B
    - Group 3: Drug C

- **Outcome Measure**: Tail-flick latency in seconds (pain response time after heat stimulus).

- **Observation Table**

Rat No.	Drug A	Drug B	Drug C
1	4.2	6.3	7.1
2	3.9	6.7	6.9
3	4.1	6.5	7.3
4	4.0	6.8	7.0
5	4.3	6.4	6.8
Mean	**4.1**	**6.54**	**7.02**

ANOVA Calculation

Step 1: Calculate Group Means
Already given above.

Step 2: Calculate Grand Mean (GM)

$$\mathbf{GM = \frac{\text{Total of all values}}{15}}$$

$$= \frac{4.2 + 3.9 + 4.1 + 4.0 + 4.3 + 6.3 + 6.7 + 6.5 + 6.8 + 6.4 + 7.1 + 6.9 + 7.3 + 7.0 + 6.8}{15}$$

$$= \frac{97.3}{15}$$

$$= \mathbf{6.49}$$

Step 3: Sum of Squares Between Groups (SSB)

$$SSB = n \sum (\bar{X}_i - GM)^2$$

Where:

- $n = 5$ (number of rats per group)
- $\bar{X}_i$ is the group mean

$$SSB = 5\left[(4.1 - 6.49)^2 + (6.54 - 6.49)^2 + (7.02 - 6.49)^2\right]$$
$$= 5\left[5.76 + 0.0025 + 0.2704\right] = 5 \times 6.0329 = 30.1645$$

Step 4: Sum of Squares Within Groups (SSW)

$$SSW = \sum (X_{ij} - \bar{X}_i)^2$$

Calculate for each group:

- Drug A:

$$(4.2 - 4.1)^2 + (3.9 - 4.1)^2 + (4.1 - 4.1)^2 + (4.0 - 4.1)^2 + (4.3 - 4.1)^2 = 0.01 + 0.04 + 0 + 0.01 + 0.04 = 0.10$$

- Drug B:

$$(6.3 - 6.54)^2 + (6.7 - 6.54)^2 + (6.5 - 6.54)^2 + (6.8 - 6.54)^2 + (6.4 - 6.54)^2$$
$$= 0.0576 + 0.0256 + 0.0016 + 0.0676 + 0.0196 = 0.172$$

- Drug C:

$$(7.1 - 7.02)^2 + (6.9 - 7.02)^2 + (7.3 - 7.02)^2 + (7.0 - 7.02)^2 + (6.8 - 7.02)^2$$
$$= 0.0064 + 0.0144 + 0.0784 + 0.0004 + 0.0484 = 0.148$$

$$SSW = 0.10 + 0.172 + 0.148 = 0.42$$

Step 5: Degrees of Freedom (df)

- Between groups: $k - 1 = 3 - 1 = 2$
- Within groups: $N - k = 15 - 3 = 12$

Step 6: Mean Squares (MS)

- $MSB = \frac{SSB}{df_{between}} = \frac{30.1645}{2} = 15.082$
- $MSW = \frac{SSW}{df_{within}} = \frac{0.42}{12} = 0.035$

Step 7: F-Ratio

$$F = \frac{MSB}{MSW} = \frac{15.082}{0.035} \approx 430.91$$

Step 8: Compare F-Ratio to Critical F-value

At **α = 0.05**, df = (2,12), the critical F-value ≈ **3.89**.

Since **430.91 > 3.89**, the result is **highly significant**.

Step 9: Conclusion

The ANOVA test confirms that the three drugs do not have equal analgesic effects. This statistical evidence supports further investigation into Drug C as a potentially more effective analgesic.