Aim: Study of Analgesic Activity by Writhing Test

References:

1. Koster, R., Anderson, M., & De Beer, E. J. (1959). Acetic acid for analgesic screening. *Federation Proceedings*, 18, 412-417.

2. Vogel, H. G. (2008). *Drug Discovery and Evaluation: Pharmacological Assays*. Springer.

Introduction:

The writhing test is a well-established method used to evaluate the analgesic (pain-relieving) activity of compounds in mice. This method involves inducing pain by administering an irritant and observing the number of writhes (constrictions of the abdomen and stretching of the body) produced. A reduction in the number of writhes compared to a control group indicates analgesic activity.

Objective:

To determine the analgesic activity of a test compound using the acetic acid-induced writhing test in mice.

Materials and Reagents:

- Mice (20-25 g, either sex)
- Test compound
- Acetic acid (0.6% solution)
- Saline or vehicle (control)
- Standard analgesic (e.g., acetylsalicylic acid)
- Syringes and needles
- Stopwatch or timer
- Animal cages
- Disposable gloves
- Laboratory coat

Procedure:

Animal Preparation

1. Acclimatize the mice to the laboratory conditions for at least one week before the experiment.

2. Fast the mice overnight with free access to water prior to the experiment.

Experimental Groups:

Divide the mice into the following groups, with a minimum of six animals per group:

1. Control group: Receive saline or vehicle

2. Standard group: Receive a standard analgesic (e.g., acetylsalicylic acid)

3. Test groups: Receive different doses of the test compound

Administration of Compounds:

1. Administer the test compound, standard analgesic, or vehicle intraperitoneally (i.p.) according to the group designation.

2. Allow 30 minutes for absorption.

Induction of Writhing:

1. Administer 0.6% acetic acid intraperitoneally to all groups to induce writhing.

2. Immediately place the mice individually in transparent observation cages.

Observation and Recording:

1. Start the stopwatch immediately after acetic acid injection.

2. Observe each mouse for 30 minutes.

3. Count the number of writhes (abdominal constrictions and stretching) for each mouse.

4. Record the data.

Calculation of Analgesic Activity:

1. Calculate the percentage inhibition of writhes for each test and standard group compared to the control group using the formula:

Percentage inhibition = (Number of writhes in control group - Number of writhes in test/standard group / Number of writhes in control group) × 100

Results and Discussion:

1. Present the data in a table showing the number of writhes for each mouse in all groups.

2. Calculate and present the mean number of writhes for each group along with the standard deviation.

3. Calculate the percentage inhibition of writhes for each test compound dose and the standard analgesic.

4. Discuss the results, comparing the analgesic activity of the test compound with the control and standard groups. A significant reduction in the number of writhes compared to the control group indicates analgesic activity.

Safety and Ethical Considerations:

1. Ensure all experimental procedures involving animals comply with institutional and national ethical guidelines for the care and use of laboratory animals.

2. Handle all animals with care and minimize their distress.

3. Dispose of all biological waste according to safety guidelines.

Conclusion:

Summarize the findings, stating whether the test compound demonstrated significant analgesic activity and how it compared to the standard analgesic.

Data Table

Group	Mouse 1	Mouse 2	Mouse 3	Mouse 4	Mouse 5	Mouse 6	Mean ± SD	% Inhibition
Control	25	28	27	30	26	29	27.5 ± 1.87	-
Standard (50 mg/kg)	12	14	13	11	15	14	13.2 ± 1.47	52.00
Test (10 mg/kg)	20	22	21	23	19	22	21.2 ± 1.48	22.91
Test (20 mg/kg)	15	17	16	18	16	17	16.5 ± 1.12	40.00