

EXPERIMENT NO.: 18

DATE: \_\_\_\_\_

**AIM: EVALUATION OF ANALGESIC EFFECT IN RAT OR MICE**

**PRINCIPLE:**

Pain is induced to a suitable animal and the response of the animal to the painful stimuli is recorded before and after administration of drugs. Analgesics drugs inhibit the perception (sensation) of the pain.

Pain is classified in to two types:

**Types of Pain:**

**a) Superficial:**

- Stimulation of skin & mucous membranes
- Fast response

**b) Deep:**

- Arises from muscles, joints, tendons, heart etc
- Slow response

**According to types of pain analgesic drug divide in to:**

**a) Peripheral (miscellaneous):**

- Causal: Treat cause pain ( E.g - antispasmodic)
- Non-causal: Treat non cause pain( E.g - Local anaesthetics for superficial tumor and Counter-irritant, apply pain that counteract or mask the original one e.g. acupuncture)

**b) Central:**

- **Narcotic: Opioids (morphine & morphine like drugs)**

Examples 1- Natural (as codeine)

2- Semi synthetic e.g. di-hydromorphine & diacetylmorphine (heroin)

3- Synthetic e.g. pethidine

4- Endogenous opiates as endorphins & enkephalins

- **Non-narcotic NSAID**

1-Aspirin

2- Paracetamol

3- Diclofenac

4- Piroxicam

5- Ibuprofen

6- Ketoprofen

**SCREENING METHODS**

**1. Narcotics:**

- A) Thermal method
  - a) Hot plate
  - b) Tail flick
- B) Mechanical method

**2. Non-narcotic:**

- A) Electrical method
- B) Chemical ( Writhing method)

**HOT – PLATE**

**MATERIAL**

Animal: Mouse or Rat

Instrument: Hot plate analgesiometer

Painful stimulus: Heat (55°C)

Drug used: Pentazocine (20 mg/Kg, i.p) or Morphine (1 – 2 mg/Kg, i.p)

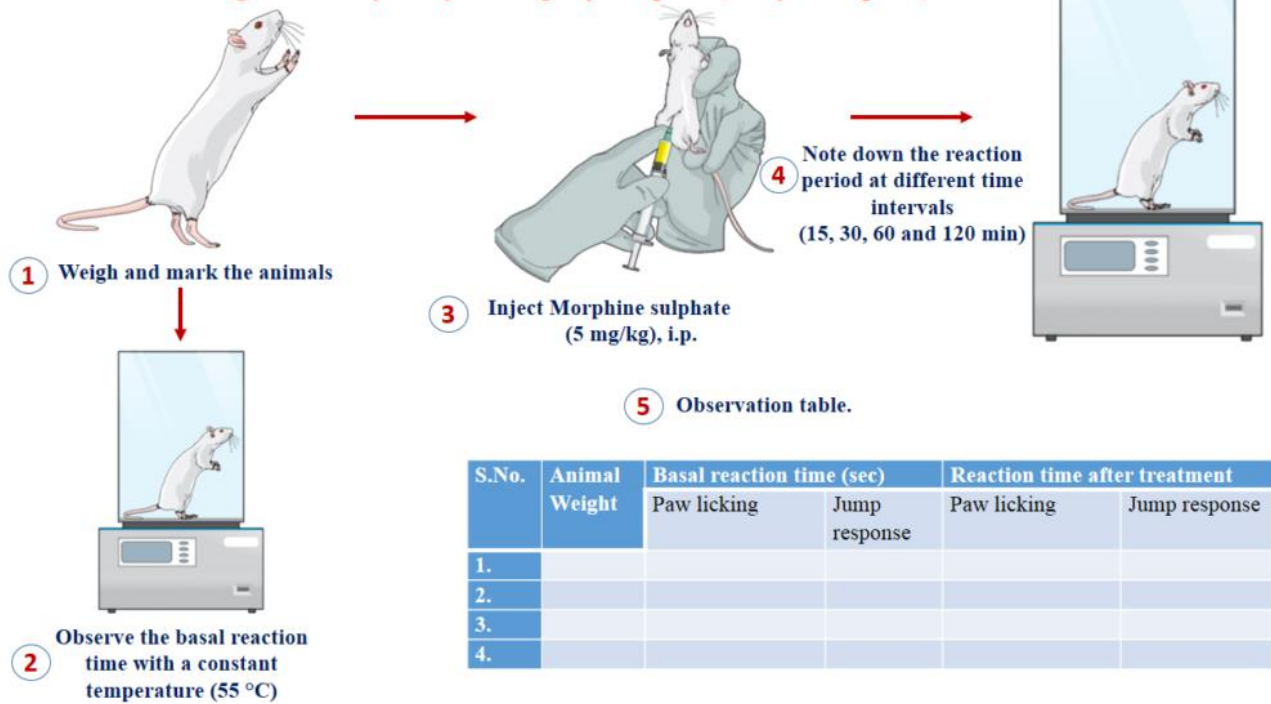
**PROCEDURE:**

- In this model prior to the experiment the hot plate was set for a temperature 55<sup>0</sup>C. Weight animal and number the rat. Take the basal reaction time by observing hind paw licking or jump response (whichever appears 1<sup>st</sup>) in animal when placed on hot plate. Normally an animal shows such response in 6-8 seconds.
- A cut off period of 15 sec is observed to avoid damage to paws. Inject Pentazocine to the animals 30 minutes prior to the recording the response. The time for licking paws or jumping in hot plate was recorded as a response, prior and 0, 30, 60, 90 120 min after administration of the drug.
- As the reaction time increased with Pentazocine, 15 seconds is taken as maximum analgesia and the animals are removed from the hot plate to avoid injury to the paws.
- Calculate percentage increase in reaction time (as index of analgesia) at each time interval

**OBSERVATION TABLES:**

Sr.No	Drug treatment dose	Time in (min)	Basal reaction time (Sec)		Reaction time (Sec) after drug administration	
			Paw licking	Jump response	Paw licking	Jump response
1.						
2.	Pentazocine 20 mg/Kg, i.p	30	2	4	7	> 10
3.		60	2	3	6	> 10
4.		90	2	4	6	> 10
5.		120	2	4	5	9

**Analgesic activity study of drugs by hot plate (Eddy's hot plate) method**



**TAIL-FLICK**

**MATERIAL**

Animal: Mouse or Rat

Instrument: Tail-flick analgesiometer

Painful stimulus: Heat (by apply a beam of light 130°C)

Drug used: Pentazocine (20 mg/Kg, i.p) or Morphine (1 – 2 mg/Kg, i.p)

**PROCEDURE:**

- Transport mice to the testing room in their home cages. Allow 15 minutes for the mice to acclimatize.
- Clean the apparatus with detergent and switch on the tail flick apparatus.
- Remove a mouse from its home cage and gently cover the mouse with a linen glove to restrain it.
- Gently hold the mouse with its tail directly under heat source and press the start button.
- Cut off period of 10 – 12 seconds is considered to prevent damage to the tail.
- Stop the timer when the mouse flicks its tail (i.e. an indication that the mouse feels pain).
- Record the latency of tail flick.
- Take at least 3 – 5 basal reaction times (trial) for each mouse at an interval of 10 minutes to confirm normal behavior of animal.
- Inject the drug and note the reaction time after 30 minutes. As the reaction time reaches 10 seconds it is considered maximum analgesia and tail is remove from the source of heat to avoid tissue damage.
- Calculate % increase in reaction time (Index of analgesia) at each time interval.

## PHARMACOLOGY AND TOXICOLOGY PRACTICAL

- Ensure that the mouse has not sustained any tissue damage before returning to its home cage.
- Clean the apparatus before testing another mouse.
- Following completion of the experimental session, switch off the tail flick apparatus.

### OBSERVATION TABLE:

Sr.No	Body weight (gms)	Drug treatment dose	Volume injected in mL	Basal reaction time (Sec.)	Reaction time 30 min. after drug treatment
1.	30	Pentazocine	0.06	5	> 10 sec
2.	35	20 mg/Kg, i.p	0.07	3	> 10 sec

### DOSE CALCULATION:

Mice weight 30 G = 30 X 10<sup>-3</sup>Kg

Dose of Pentazocine is 20 mg/Kg

1 Kg animal required ----- 20 mg dose

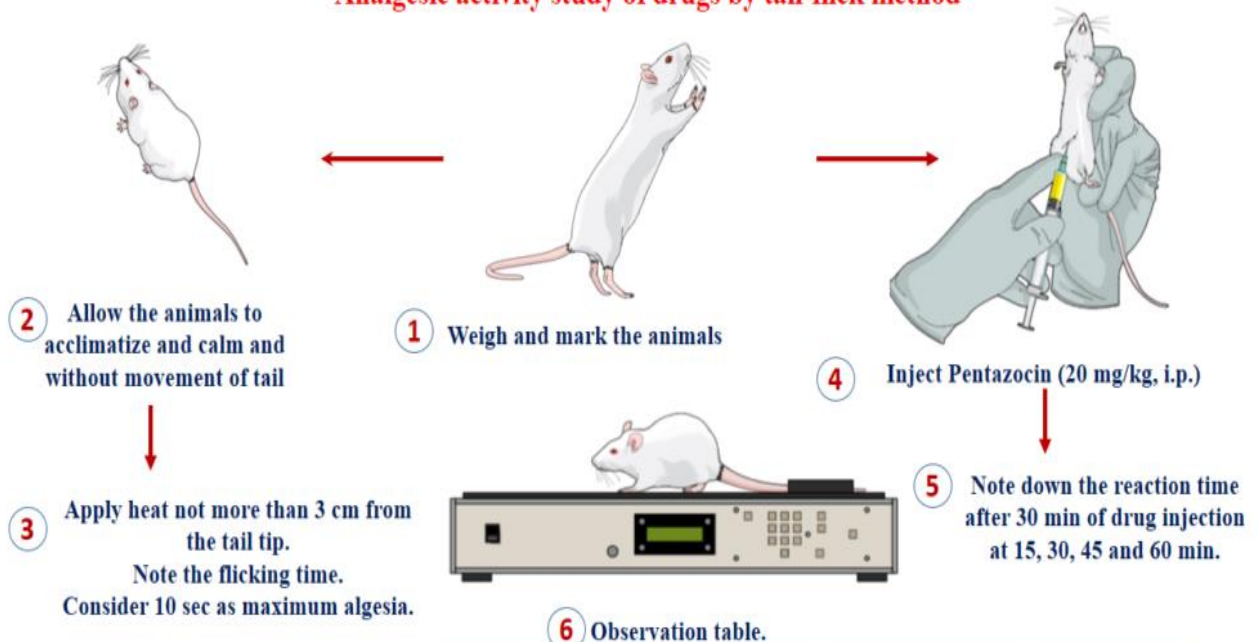
30 X 10<sup>-3</sup>Kg animal required ----- (?) = 0.6 mg

Stoke solution = 10 mg/mL

10 mg drug required ----- 1 mL dose

0.6 mg drug required ----- (?) = 0.06 mL dose

### Analgesic activity study of drugs by tail-flick method



Sl. No.	Body Weight	Treatment	Basal reaction time (sec)	Reaction time after 30 min of drug treatment

# PHARMACOLOGY AND TOXICOLOGY PRACTICAL

## WRITHING METHOD

### MATERIAL

Animal: Mouse

Painful stimulus: Acetic acid (1% V/V, 1 mL/Kg, S.C)

Drug used: Aspirin (200 mg/Kg)

### PRINCIPLE:

The painful stimulus is induced by IP injection of an irritant substance (e.g. acetic acid)

### Writhing:

Stretching of the body, withdrawing of the limb, retraction of the abdomen & the stomach touches the ground

### PROCEDURE:

- Weight and number the animals
- Divide the animals in to control and test groups (n=5).
- **Control group:**
  - Administered appropriate volume of acetic acid solution to the control group.
  - Note the onset of writhing. Record the number of abdominal contractions, trunk twist response and extension of hind limb as well as the number of animals showing such response during period of 10 min.
- **Test group (Drug treated):**
  - Inject morphine and after 15 min. of injection, administered acetic acid solution to the animals. Note the onset and severity of writing response as said above.
  - Calculate the mean writhing scores in control and morphine treated groups. Note the inhibition of pain response by morphine.

### CALCULATION TABLE:

Animal group	No. of animal	Body weight	Drug	Volume injected in mL	Number of writing	Mean value of writing	% inhibition of pain
Control	1	25	Acetic acid ( 1% V/V, 1 mL/Kg, S.C )	0.25	33	32.8	0
	2	32		0.32	37		
	3	30		0.3	26		
	4	34		0.34	35		
	5	27		0.27	33		

## PHARMACOLOGY AND TOXICOLOGY PRACTICAL

Test	1	26	Aspirin (200 mg/kg, i.p)	0.26	10	10.2	68.9
	2	33		0.33	12		
	3	31		0.31	11		
	4	32		0.32	10		
	5	34		0.34	08		



**SIGNATURE OF TEACHER**