EXPERIMENT NO:-16 **DATE**

AIM: To evaluate the analgesic activity of drug using central and peripheral methods

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:

THEORY

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 & encephalins

- Non-narcotic NSAID

  1-Aspirin

  2- Paracetamol

  3- Diclofenac

  4- Piroxicam

  5- Ibuprofin

  6- Ketoprofin

SCREENING METHODS

1. **Narcotics**:

A) Thermal method

a) Hot plate

b) Tail flick

B) Mechanical method

a. Pressure on tail is given by a plunger. When threshold required is twice the normal, it is positive. (Morphine 6-9 ma/1-g).

b. Screw dense method.

c. Tail clip method.

2. Non-narcotic:

A) Electrical method

a) Pododolorimeter: cage has metallic floor, voltage required to make animal to cry and struggle is noted before and after administration of drug.

b) Recrodolorimeter: A small electrode is inserted into rectum of animal and crying or jumping is noted before and after administration of drug. Advantage over other method is that here voltage required is very less.

B) Chemical (Writhing method)

HOT – PLATE

AIM: To evaluate the analgesic effect of drugs by Hot Plate method.

REQUIRMENT

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)

PRINCIPLE:-

Animal is placed on hot-plate and time for jumping from plate is noted before and after administration of drugs. In this method Eddy's Hot Plate is used. The basal reaction time is recorded. Here, the reaction may be hind paw licking or jump response. Hand appears within 4-6 sec. and after two to three sec. jumping may start. One has to observe both these response before and after administration of drug like morphine.

PROCEDURE:

- In this model prior to the experiment the hot plate was set for a temperature 55^0C. Weight animal and number the rat. Take the basal reaction time by observing hind paw licking or jump response (whichever appears 1^{st}) 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

HOT PLATE

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.	Pentazocine 20 mg/Kg, i.p					
2.						
3.						
4.						
5.						

TAIL-FLICK

AIM: To evaluate the analgesic potency of drug by thermal method.

REQUIRMWNT:-

Instrument: Analgesiometer

Animal: Mouse

Painful stimulus: Pethidine.

Drug used: Aspirin (200 mg/Kg)

PRINCIPLE:

The animal is exposed to the noxious stimulus (heat, electricity or chemical) and the time taken to produce response (Flickering of tail or jumping) is recorded. The analgesiometer consists of an electrically heated nicrome wire. On the either side of nicrome wire there are metallic square tubes through which cold water is circulated. Thus only part above nicrome wire is heated while other part is not affected. The circulation of cold water prevents dispersion of teat in the surrounding area. The heated nicrome wire (55° C) acts as noxious stimulus and the time taken to produce the effect is noted down.

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.
- 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.

ANALGESIOMETER

OBSERVATIONS:

When the drug pethidine is given the reaction time is increased to 16 sec. (test) from 10 sec. (control).

Animal Nos.	Basal Reaction Time (Sec.)					Reaction Time (Sec.) after drug administration		
	1	2	3	4	5	10 min	20 min	30 min
01								
02								
03								
04								

DISCUSSION:

Various analgesics- Narcotics-morphine, pethidine, heroin etc.
Non-narcotics-salicyiates, phenyibutazone., i.p)

WRITHING METHOD

AIM: To evaluate the analgesic potency of drug by writhing method

REQUIRMWNT:-

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.

WRITHING OF MICE

OBSERVATION TABLE:

DISCUSSION:

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			
	2	32		0.32			
	3	30		0.3			
	4	34		0.34			
	5	27		0.27			
Test	1	26	Aspirin (200 mg/kg, i.p)	0.26			
	2	33		0.33			
	3	31		0.31			
	4	32		0.32			
	5	34		0.34			

QUESTIONS-

1. Enlist the methods used for screening of analgesics.
2. Enlist the instrument used for screening of analgesics

3. Classify analgesic drugs.
4. What is the mechanism of action of aspirin and pentazocin?
5. What is writhing?
6. What is noxious stimulus?
7. What is temperature of nicrome wire to produce noxious stimulus?
8. What is the purpose of circulation of cold water in the analgesiometer?
9. How much a cut off period for avoid damage to paws on hot plate?