Chapter – VI

STUDIES ON ANALGESIC ACTIVITY
- Introduction
- Experimental methods
- Pharmacological screening:
  - Ethanolic extract of the seeds and leaves of *Ricinus communis*
- Results and discussions
ANALGESIC ACTIVITY

• Introduction

The naked nerve endings are the sensing zones for pain found in almost every tissue of the body. The pain impulses are transmitted to the CNS by two fibre systems. The nociceptor system is made up of small myelinated A δ fibres 2-5 μ in diameter which conduct at 12- 30 m/s. The unmyelinated C fibres 0.4 - 1.2 μm in diameter conduct at slower rate of 0.5 -2 m/s and also called dorsal root fibers. A δ fiber terminates in laminae I and V, where as the dorsal root C fibres terminate in laminae I and II. There is evidence that the synaptic transmitter secreted by primary afferents, subserving mild pain is glutamate and transmitter subserving severe pain is substance P. The synaptic junction between the peripheral nociception fibres and the dorsal horn cells in the spinal cord are sites of considerable plasticity and hence called gate. Collateral branches from the touch fibres in the dorsal column enter the substantia gelatinosa and it has been postulated that the impulses in these collaterals or interneurons on which they end inhibit transmission from dorsal root pain fibres to spinothalamic neurons. The primary mechanism involved may be synaptic inhibition at the endings of the primary conductors that transmit the pain impulses.

Some of the axons of the dorsal horn neuron end in the spinal cord and brain stem. Others enter the anterolateral systems including lateral spinothalamic tract. Some of the ascending fibres project to the ventral posterior nuclei, which are specific sensory relay nuclei of thalamus and from there to cerebral cortex. Pain activates these cortical areas: SI, SII and the cingulated gyrus on the side opposite the stimulus. Fast pain is due to A δ pain fibres and slow pain is due to C-pain fibres.
The adequate stimulus for pain receptors is not specific as that for others. Pain receptors respond to a variety of strong stimuli factors / effects originating from thermal, electrical, mechanical and chemical changes.

It is suggested that the pain is chemically modulated and that stimuli in common the ability to liberate a chemical agent that stimulates nerve endings which may be due to ATP. It opens ligand gated channels on sensory neurons via P2x receptors. Capsaicin is a component responsible for burning pain. A capsaicin receptor that is non selective ion channel permits flow of Na$^+$ and Ca$^{2+}$ into nociceptive neurons when activated producing depolarization. This channel is activated by warmth.

Pain is as perception alone which does not require cortex. Nociception is the mechanism by which nervous peripheral stimuli are transmitted to the central nervous system. Pain is subjective not always associated with nociception. Polymodal nociceptors (PMN) are the main types of peripheral sensory neurons that responds to various stimuli. Chemicals stimulate by acting on PMN to cause pain include bradykinin, 5-HT and capsaicin. PMN are sensitized by prostaglandins which explains analgesic effect of aspirin like drugs particularly in inflammation$^{35}$.

- **Post injury and Neuropathic pain**

Post injury pain persists after injury. The stimuli in that injured region produce exaggerated response (hyperalgesia) and stimuli such as touch cause pain (allodynia). If the nerves are damaged the pain persist after injury heals (neuropathic pain).

In post injury and neuropathic pain, there is increased sensitivity of peripheral pain receptors due to local release of sensitizing substances. There is also increased transmission at the synaptic junction between the first and the second order neuron in dorsal horn.
There is increased activity of pre-synaptic NMDA receptors on primary afferents with increased release of substance P.

Afferent fibres incase of visceral structure reach C.N.S. via sympathetic and parasympathetic pathways. There are visceral afferents in the facial, glossopharyngeal and vagus nerves in the thoracic and upper lumbar dorsal seeds and sacral seeds. In the C.N.S. visceral sensation travels along the same pathways as somatic sensation in the spinothalamic tracts. Thalamic radiations and cortical receiving areas for visceral sensations are intermixed with somatic receiving areas.

Visceral pain initiates reflex contraction of near by skeletal muscles. It is marked when visceral inflammatory process involve peritoneum.

- **Nociceptor**
  
  An orphan receptor that causes hyperalgesia rather than analgesia.

- **Chronic pain syndromes**
  
  Neuropathic pain in humans has various forms. In causalgia, there is spontaneous, burning pain long after seemingly trivial injuries. The pain is accompanied by hyperalgesia and allodynia. Nerve injury leads to sprouting and eventual over growth of noradrenergic sympathetic nerve fibres into dorsal root ganglia of the sensory nerves to injured area. Sympathetic discharge then brings no pain. Alpha adrenergic blockade produces relief of causalgia-a type of pain in humans.

  Spontaneous pain may be generated at the level of thalamus for eg in thalamic syndrome; there is damage to the posterior thalamo geniculate branch of posterior cerebral artery.
• Experimental methods - Analgesic effects

Many methods are available for evaluation of analgesic effect. In all the methods, one or other type of stimulus is applied to produce pain reaction. The methods can be classified based on the type of stimulus used.

1. Thermal stimulus
   a) Hot plate test
   b) Radiant heat method using analgesiometer or electric bulb as the source.

2. Mechanical stimulus
   a) Tail clip
   b) Randal Sellito test

3. Chemical stimulus
   a) Writhing by acetic acid or phenylquinone

4. Electric stimulus
   a) Pododolorimeter
   b) Rectodolorimeter

Of the methods available, the Randall Sellito test and the chemically induced writhing tests are used for the evaluation of the analgesic activity of peripherally acting drugs. The other methods enumerated are mainly meant to evaluate drugs that act by central mechanisms. However, the tail clip, tail flick and hot plate methods could be employed for screening of non-narcotic drugs.
PHARMACOLOGICAL SCREENING—
ANALGESIC ACTIVITY

- Ethanolic extracts of the seeds and leaves of *Ricinus communis*
  - Preparation of extracts
    
    The preparation of ethanolic extracts from the leaves and seeds of *Ricinus communis* was discussed under chapter-III (chemical investigations) and chapter-IV (studies on hepatoprotective activity). These extracts were used to evaluate analgesic activity of the seeds and leaves of *Ricinus communis*.

- Selection of Method
  
  Analgesic activity was evaluated by acetic acid induced Writhing method\(^{36}\).

- Selection of Materials
  
  All the materials used for this experiment were of analytical reagent grade. Acetic acid was procured from Loba Chemical Co., Bombay and Aspirin sample was obtained as a gift from Astra zenica, Bangalore, India.

- Selection of animals
  
  Healthy albino mice of either sex weighing about 25-30 g obtained from Kshema Breeders, Deralakatte, and Mangalore, India were used for the study. They were housed in polypropylene cages in an adequately ventilated room. The mice were allowed standard feed pellets supplied by Hindustan Lever Co., Bombay and water *ad libitum* throughout the course of the study.
 **Preparation of Tween 80 (1%)**

Stock solution of Tween 80 was prepared by triturating 1g of Tween 80 in distilled water and made up to 100 ml. It was used for suspending the test compound and standard drug.

 **Selection of the dose**

As the ethanolic extracts from the seeds and leaves of *Ricinus communis* were found to be safe upto a dose of 4000 mg/kg body weight. A dose of 250 mg/kg body weight was selected for the study of analgesic activity of the seeds and leaves of *Ricinus communis*.

 **Analgesic activity**

Healthy albino mice of either sex weighing 25 –30 g were used for the study. The animals were divided into 4 groups of 6 animals each.

**Group I**

Animals were administered with 0.5 ml of 1% solution of Tween 80 and served as control.

**Group II**

Animals served as positive control and received acetyl salicylic acid (Aspirin) (50 mg/kg body weight, p.o.) as a suspension in Tween 80 (0.5 ml of 1% solution).
Group III

Animals were treated with ethanolic extract (250mg/kg body weight, p.o.) of the seeds of *Ricinus communis* obtained by the author as a suspension in Tween 80 (0.5 ml of 1% solution).

Group IV

Animals were treated with ethanolic extract (250mg/kg bodyweight, p.o.) of the leaves of *Ricinus communis* obtained by the author as a suspension in Tween 80 (0.5 ml of 1% solution).

Further the animals in all the groups were administered with 1ml/100g body weight of 0.6% v/v acetic acid after 45 minutes.

The analgesic activities of the extracts were assessed by the acetic acid induced writhing test. The writhing movements such as extension of hind limb, abdominal constriction and trunk twisting were observed and counted for 30 min. Aspirin was used as a standard drug.
RESULTS AND DISCUSSION

The results of acetic acid writhing test in mice showed a significant decrease in number of wriths in ethanolic extracts of both seeds and leaves of *Ricinus communis* suggesting peripheral analgesic effect.

In the acetic acid induced writhing method the standard analgesic drug (Aspirin 50mg/ kg, p.o.) as well as the test drugs of ethanolic extracts of the seeds and leaves of *Ricinus communis* obtained by the author in doses of (250 and 250 mg/kg, p.o.) showed a significant reductions in the number of writhing in mice as compared to the control mice. Aspirin (50mg/kg) showed maximum of 72.73% of inhibition of writhing, where as the ethanolic extracts of the seeds and leaves of *Ricinus communis* (250 and 250mg/kg) produced 56.82% and 68.18% of inhibition of writhing respectively (Table 10). The results indicate that the ethanolic extract of the seeds / leaves are more or less equally successful in serving the cause of pain relief.
Table-10
Analgesic activity of ethanolic extracts of the seeds and leaves of *Ricinus communis*.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose</th>
<th>Mean writhing per 30 min ± SEM</th>
<th>Inhibition %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Tween 80, p.o.</td>
<td>88± 0.8</td>
<td>…………</td>
</tr>
<tr>
<td>Aspirin</td>
<td>50 mg/kg, p.o.</td>
<td>24±1.63*</td>
<td>72.73</td>
</tr>
<tr>
<td>Ethanol extract (seeds)</td>
<td>250 mg/kg, p.o.</td>
<td>38±1.55*</td>
<td>56.82</td>
</tr>
<tr>
<td>Ethanol extract (Leaves)</td>
<td>250 mg/kg p.o.</td>
<td>28.0±0.43*</td>
<td>68.18</td>
</tr>
</tbody>
</table>

*p < 0.001 compared to control

Values are mean ±SEM, of six animals in each group.

Data was analysed by unpaired ‘t’ test.

**Fig 10 : Percentage inhibition of acetic acid induced writhing in mice**

- 50 mg/kg, p.o.of the standard drug
- 250 mg /kg, p.o. of ethanolic extract of the seeds of Ricinus communis
- 250 mg/kg p.o.of ethanolic extract of the leaves of Ricinus communis
REFERENCES


