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Review Article

A Detailed Review on Nociceptive Models for the Screening of Analgesic Activity in Experimental Animals

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Abstract: Pain is an unpleasant sensation, which informs about any abnormality in either body or part of it. Pain may be either physical or mental depending on its source of origin. Generally, mental or psychic pain is treated with antipsychotic agents, which include, antidepressant, anti-anxiety and anti manic drugs. However, the pain generated by physical stimuli may be treated with analgesic and anti-inflammatory medicine. Analgesic is those drugs or agent, which reduce or block the sensation of pain temporarily. Several synthetic and plant origin analgesic are being tested for their efficacy and potency on different animal model including hot plate, tail flick, tail clip, cold pain, filament pain, tail immersion technique, acetic acid induced writhing test, formalin induced writhing test. Many plant extract have been proved to be analgesic just by evaluating through any one of these model stated, but the present review is based on the detail working principle and constructional detail of all the possible models generally available in pharmacology laboratory. This review also provides the principles of several new models like, tooth pulp, formalin induced writhing, monkey shock test so that they should be used in future.

Keywords: Analgesia, Analgesic Models, Nociception, Hot Plate, Tail Flick

1. Introduction

No one likes the pain but it is one of the most important defensive mechanisms in our body, which provide us signal about the abnormality. Depending upon the severity and intensity of threshold, pain has classified into two main classes either acute or chronic. When pain occurs in quick succession and disappears after few hours or day with or without medication then it is acute type and one the other hand if it develops after long time and goes slowly or incompletely than it is a chronic type. From the point or origin to the point of receiving, sensation of pain, involve central as well as peripheral nervous system. Alleviation of pain depends on several factors like its type, its origin point, and causes behind that pain. Neurogenic pain may be arise due to anxiety, depression, mania, epilepsy, seizer, phobia and many more, so their treatment need application of neurotherapeutic agents which act on serotonin/nor epinephrine reuptake inhibitor while the normal pain such as, body ach, arthritic pain, inflammatory pain, traumatic pain need normal analgesic medication like non steroidal anti inflammatory agents [1] Opioids are most widely utilized analgesic drug worldwide, thus it become drug of choice for pain. Mechanism is to bind on opioids receptor in the central nervous system (CNS) to produce the effect just like endogenous peptide neurotransmitters like endorphins, encephalin, and dynorphins [2].

Opioids are obtained from the juice of opium poppy, which is a natural plant derived source. In present scenario many research papers are based on analgesic associated with anti-inflammatory action of many plant [3]. Unfortunately, they use either one or two evaluation models for screening of analgesic activity, which is inappropriate with the research. Evaluation models of analgesic drug are based on many parameters like state and mood of experimental animal, condition of animal during experiments, evaluation models

previously decided to use, and above all the principle behind the specific models and causation or stimulation of pain in animal just to evaluate the efficacy and potency of plant extract. Present review is based on different models available for the evaluation of analgesic activity in pharmacology lab along with their basic principle and instrumentation of apparatus.

Table 1. *Basic terminology used in pain management [1, 4].*

Serial No.	Terms	Definition
01	Pain	Unpleasant sensation that may be arise due to any reason
02	Noxious Stimulus	Substance, which generate pain in experimental animal, during the evaluation process.
03	Receptor	Macromolecule presents either on surface of inside the cell just to recognize the signaling molecule.
04	Threshold	The minimum intensity of stimulus necessary to cause pain without damaging the tissue [4].
05	Analgesic	Agent, which inhibit or block the sensation of pain on temporary basis.

2. Mechanism of Analgesic Drugs

The perception of pain is due to activation of nociceptive receptor by the neurotransmitters. Three receptor has been identified for the pain perception, mu, kappa, and delta. They initiate the synthesis of either prostaglandin I or prostaglandin II or sometime both. Analgesic dugs block them either selectively or none selectively to the COX-II receptor. Opioids relieve pain by increasing the threshold at spinal cord level, thus individual may withstand with higher level of pain [4, 5].

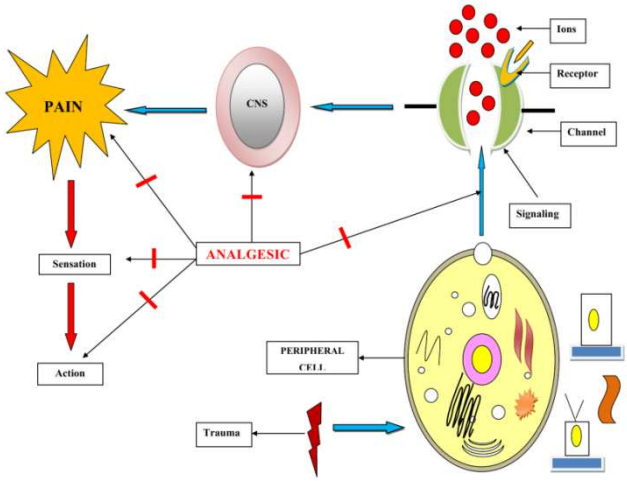

Figure 1. *Mechanism of Action of Analgesic Drugs.*

3. Evaluation Models for Analgesic Activity

Evaluation is essential steps in the development and clinical trial of any analgesic drugs. The drug or plan extract is being tested on different animal models available in laboratory. Not all models are based on same principle, thus one has to be very selective and accurate with the selection of suitable model [5]. In literature survey of about random and open access journal I have found the surprising result that most of the researchers followed only one to two models for evaluation purpose, and another fact came out through this survey that only two models were widely used- hot plate method and acetic acid induced writhing test [6-10].

Table 2. *Different models for the evaluation of analgesic activity in experimental animals [2].*

S. N.	Model	Principle	Utility
01	Tail Flick Method	Thermal Stimuli	Widely Used
02	Paw Withdrawal Test	Thermal Stimuli	Widely Used
03	Hot Plate Method	Thermal Stimuli	Widely Used
04	Cold Stimuli Model	Thermal Stimuli	Rarely Used
05	Tail Immersion Method	Thermal Stimuli	Occasionally
06	Tail Clip Method	Mechanical Stimuli	Occasionally
07	Strain Gauges	Mechanical Stimuli	Rarely Used
08	Von-Fery Filament	Mechanical Stimuli	Rarely Used
09	Electrical Stimulation in Tail	Electrical Stimuli	Rarely Used
10	Grid Shock Test	Electrical Stimuli	Rarely Used
11	Stimulation in the Tooth Pulp	Electrical Stimuli	Rarely Used
12	Monkey Shock Test	Electrical Stimuli	Rarely Used
13	Food Shock Test	Electrical Stimuli	Rarely Used
14	Formalin Test	Chemical Stimuli	Widely Used
15	Acetic Acid Writhing Test	Chemical Stimuli	Widely Used
16	Stimulation on Hollow Organ	Chemical Stimuli	Rarely Used

3.1. Hot Plate Method

Hot plate method of analgesic evaluation is based on the thermal stimuli principle. Animal used in this procedure firstly introduced to the pain by applying heat to their paw [6-7, 11]. This will cause pain and after few minute rats will start, licking their paw and trying to stand by one leg for moment and then inject the medicine or plan extract, which is to be evaluated. The hot plat temperature must be maintained at 55°C consistently [12-14].

Systematically procedure is as follows-

1. Weigh and number the mice/rat used for experiment.
2. Dived animals into three groups- (1) Reference (2) Control (3) Experimental Groups.
3. Note the reaction time of rat by licking or jump response in animal after placing them on hot plate.

4. A cut off time will be about 15 sec to avoid unnecessary pain and damage.
5. Inject the drug (Plant extract) on experimental animal and allow the drug to be absorbed, and again place them on hot plate and note down the basal reaction time.
6. Compare response time before and after medicine insertion.
7. Repeat procedure if satisfactory result or response is not received.

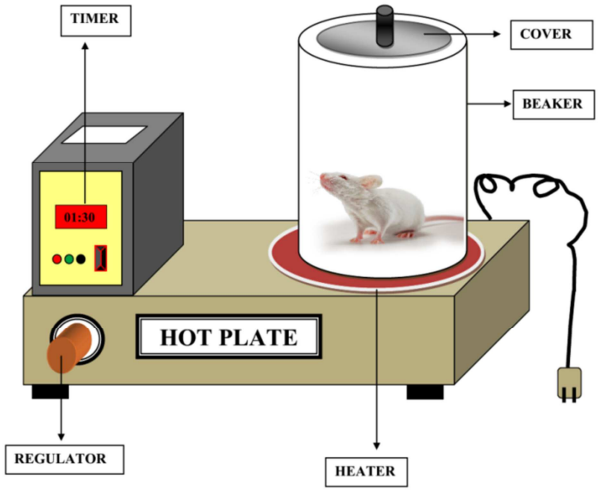

Figure 2. *Explanatory diagram of Hot Plate Analgesiometer.*

3.2. Tail Flick Method

Tail flick model is second widely used animal model for the evaluation of analgesic activity in either rat or mice [5]. Method on the simple principle as tail of mice comes with contact to heat or thermal stimuli it will try to remove his tail or flick his tail from the stimuli source. It shows the normal reaction time for the pain perception and considered as the end point. This behaviour is also applicable for human [15-16]. After the tail flicking by rat, they are treated with given analgesic medication and then again, their response time is noted. If that drug has analgesic, property there will be delay in response time [17]. Generally, rat show response in 3 to 5 seconds, if it takes more than 10-12 second than those rats will remove from experiments to avoid further damage [18-20].

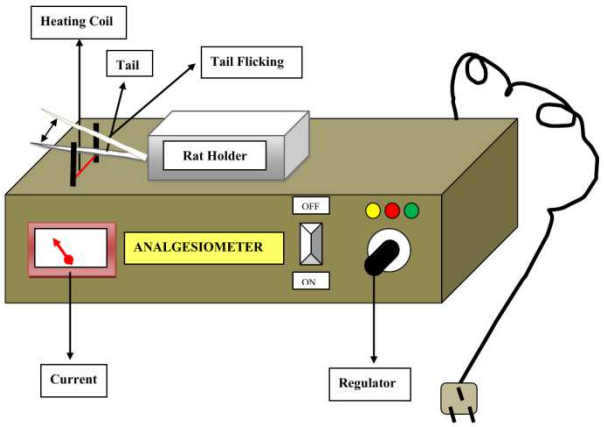

Figure 3. *Explanatory diagram of Tail Flick Analgesiometer.*

Procedure [5]

1. Weigh and number the mice/rat used for experiment.
2. Dived animals into three groups- (1) Reference (2) Control (3) Experimental Groups.
3. Note the reaction time of rat by flicking its tail from thermal stimuli source.
4. A cut off time will be about 10-12 sec to avoid unnecessary pain and damage.
5. Inject the drug (Plant extract) on experimental animal and allow the drug to be absorbed, and again place them to thermal stimulus source and note down the basal reaction time.
6. Compare response time before and after medicine insertion.

3.3. Tail Immersion Method

Analgesic activity was also checked in wistar albino rats by the caudal immersion [17]. Tail immersion method is very much similar to the tail flick method as both involve heat stimuli for causation of pain, but differ in type of heat [21-22]. In tail, flick heat source is coil and in tail immersion, hot water is used as stimulus. Rests of the procedure are same. The experimental animal were kept in cage and only one third of tail is allowed to come out side and then deepen in 51-55°C hot water bath until rat withdraw its tail, this is reaction time to stimuli and it is noted down. The cut out time is about 180 sec to prevent injury [23-25].

Procedure:

1. Weigh and number the mice/rat used for experiment.
2. Dived animals into three groups- (1) Reference (2) Control (3) Experimental Groups.
3. Note the reaction time of rat by dipping its tail in 51-55°C warm water.
4. A cut off time will be about 120 sec to avoid unnecessary pain and damage.
5. Inject the drug (Plant extract) on experimental animal and allow the drug to be absorbed, and again place them to thermal stimulus source and note down the basal reaction time [26-29].
6. Compare response time before and after medicine insertion.

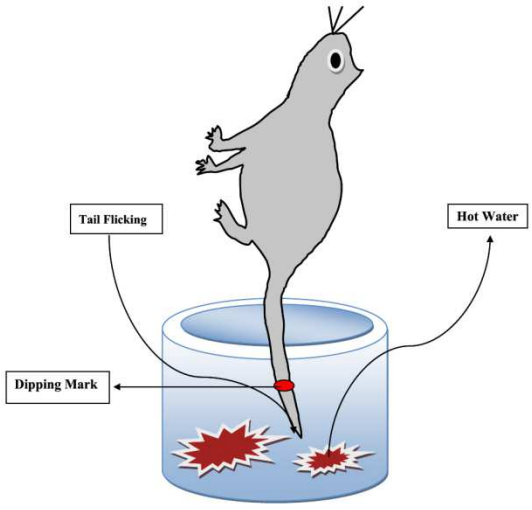

Figure 4. *Explanatory diagram of Tail Immersion.*

3.4. Haffner's Tail Clip Methods

Haffner gave this method of analgesic evaluation in around 1929. According to this procedure if the base of tail is clipped with any object and tightly than there will be a generation of pain in tail, thus mice will start biting that portion of its tail. By using this simple yet important phenomenon, we may apply drug to be evaluated and record the response weather it bite tail quickly or in latency [30-31]. If given drugs have analgesic potential than rat will not bite its tail so frequently. Mice that do not show any response within 15 seconds will reject from experiment [32-34].

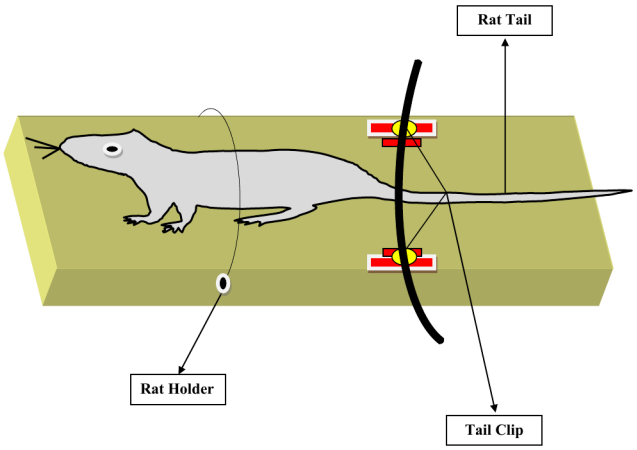

Figure 5. *Explanatory diagram of Tail Clip Analgesiometer.*

3.5. Grid Shock Test

In this model, mice are used for the evaluation purpose. They were place in the chamber before the experiment so that they may become familiar to the assembly [10]. This assembly is made of a plastic chamber, which is equipped with the wired mesh at bottom. The stimulus was given in the form of electrical photons, on 30-32 cycles per second bases for maximum of 02 minutes to avoid any injury [13]. After initiation of current flow, mice try to escape or jump from that surface. Then this activity may be record either by using oscilloscope or by simply slow motion video recorder. The same activity is repeated after the injection of drug at every 15-minute interval [16].

Procedure:

1. Weigh and number the mice/rat used for experiment.
2. Dived animals into three groups- (1) Reference (2) Control (3) Experimental Groups.
3. Note the reaction time of rat by applying current at grid.
4. Inject the drug (Plant extract) on experimental animal and allow the drug to be absorbed, and again place them to thermal stimulus source and note down the basal reaction time.
5. Compare response time before and after medicine insertion.

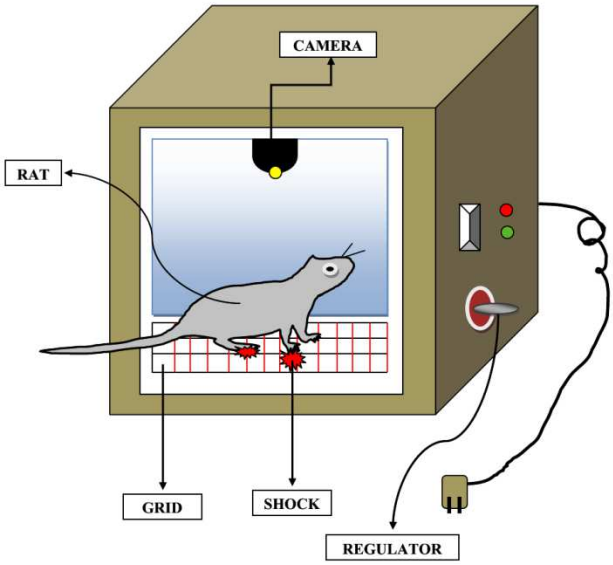

Figure 6. *Explanatory diagram of Grid-Shock Analgesiometer.*

3.6. Acetic Acid Writhing Test

Painful stimulation can also produced by chemical substance as the evolutionary method [35]. For this purpose generally acetic acid, phenylquinone, bradykinin is used by injecting them into peritoneal cavity of rat/mice [36-38]. When chemicals injected to them, they start writhing due to pain. Pain is also complied with abdominal cramp, discomfort, twisting of hind legs and extension of body. These symptoms are used as signal of pain. If given analgesic drug reduce these symptoms of pain, this will be considered effective. Narcotic and non-narcotic analgesic is used for the relieving of pain caused by the writhing [39-41].

Procedure:

1. Weigh and number the mice/rat used for experiment.
2. Dived animals into three groups- (1) Reference (2) Control (3) Experimental Groups.
3. Administer appropriate volume of acetic acid solution to the experimental group.
4. Note the onset of writhing. Record the number of abdominal contraction, turn and twist response and extension of limb for the duration of 10 minutes.
5. Inject the drug (Plant extract) on experimental animal and allow the drug to be absorbed, and again repeat the procedure by injecting acetic acid
6. Compare response time before and after medicine insertion.
7. Antinociceptive activity can also be expressed by percent maximal possible effect (%MPE)

$$\% \text{ MPE} = \text{Mean writh in treated group} - \text{Mean control writh} / \text{cut off time (Sec)} - \text{mean control writh} \times 100$$

3.7. Formalin Induced Writhing

Formalin induced writhing in rat is considered for the chronic pain evaluation model. Formalin is also used in the evaluation of anti-inflammatory drug [42]. Here in the test of

analgesic drug the 37% solution of formaldehyde is injected in the front paw of rat. Few minute after the injection its paw become swollen and pain start. After the initiation of pain rat start licking and biting its paw, that is recorded as the indication, and then different parameter are noted down like walking of rat with its full paw or jumping behavior to protect its paw from pain [43-45]. This all indication repeated after injection of analgesic drug.

3.8. Electrical Stimulation in Tail

Electrical stimulation on tail also gives satisfactory result in evaluation of analgesic drugs. When an electrode is inserted subcutaneously in tail of rat and connected to the electric source which supply very nominal current of about 40-50 V. when current is supplied rat initiate the reflex action, this reflex initiation time is recorded and calculated for the analgesic potency when analgesic drug is injected in rat. This process has one disadvantage that animal may feel more pain than usual and some time death may be possible [10, 13, 16].

3.9. Stimulation of Hollow Organ

All the materials and methods described earlier used to measure the intensity of pain and efficacy of analgesic drug of peripheral pain and sometime central pain, this model is truly made for the visceral pain [46]. The algogenic substance like formalin, acetic acid is directly injected into the hollow organ of animal, this produce complex pain like body stretching and contraction of body. This method is important because it involve the assessment of that type of pain, which are associated with internal organ, as sometime the actual reason of internal pain is not known. The analgesic drug used for such pain may easily evaluated by this procedure.

3.10. Tooth Pulp Stimulation

Tooth pulp stimulation as name indicates involve the removal of tooth pulp from experimental animal, in this case rabbit is used as in vivo animal. Firstly, rabbit of around 3 kg has been anesthetized with 15 mg/kg fentanyl-citrate intravenously than pulp chamber will be removed up to visualization of gingival line so that the root sensitivity may achieve. This whole process is done by a sterilized driller and than an electrode is inserted into that cavity and a small frequency of current is applied with 0.2 mA and slowly increase if the licking is not appear. Sometime the current velocity is gradually increased and then decreased to active the exact threshold of the current. To get accurate result this procedure is repeated 3-4 times and finally the drug to be evaluated is injected intravenously to see the potency and efficacy [2, 5]

3.11. Monkey Shock Test

Weiss gave this test in 1958 and then used by several researcher with little modification. In this method monkey is allowed to seat in the chair and electrical current is delivered through coulboum instrument programmable shocker through the electrode, which is attached to the shaved tail of monkey. The intensity of current change from zero to four-mili ampere for about 29 steps. The monkey presses a bar to disturb the flow of current and to get relief from the pain generated by that current. A point of pain is stabilized so that the evaluation of analgesic activity of drug may be determined by change in maximum level of median shock intensity that can be withstood by animal [2].

4. Conclusion

Based on literature survey and detailed study of analgesic evaluation models it is concluded that every model is based on different parameter and principle so not all analgesic medicine can be evaluated on same model. Apart from this is observed that hot plate, tail flick and formalin induced writhing test is mostly used models widely used for the general pain but not for specific pain like trauma, tumor or incision pain so we must find the reason of pain than we should choose analgesic models.

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