CHAPTER - 9

STUDIES ON ANALGESIC AND ANTIPYRETIC ACTIVITY

9.1. Introduction

Acute pain, for the most part, results from disease, inflammation, or injury to tissues. It is immediate and usually of a short duration. Acute pain is a normal response to injury and may be accompanied by anxiety or emotional distress. The cause of acute pain can usually be diagnosed and treated. Chronic pain is continuous pain that persists for more than 3 months, and beyond the time of normal healing. It ranges from mild to severe and can last weeks, months, or years to a lifetime. The cause of chronic pain is not always evident, although it can be brought on by chronic conditions such as arthritis and fibromyalgia. Chronic pain can often interfere with a patient's quality of life, sleep, pain and the diseases of the bones, muscles, joints, and skin, which affect millions of peoples. Most of these diseases are chronic and may cause lifelong pain. In certain cases, such as with some rheumatic diseases, the sources of pain may include inflammation of the synovial membrane (tissue that lines the joints), the tendons, or the ligaments; muscle strain; and muscle fatigue. A combination of these factors contributes to the intensity of the pain. Muscle inflammation characterizes other painful disorders such as polymyositis (characterized by inflamed and tender muscles throughout the body, particularly those of the shoulder and hip) and dermatomyositis (characterized by patchy red rashes around the knuckles, eyes, and other parts of the body, along with chronic inflammation of the muscles[1].

An analgesic (colloquially known as painkiller) is any member of the diverse group of drugs used to relieve pain (achieve *analgesia*). This derives from Greek *an-*, "without", and *-algia*, "pain". Analgesic drugs act in various ways on the peripheral and central nervous system; they include paracetamol (acetaminophen), the nonsteroidal anti-inflammatory drugs (NSAIDs) such as the salicylates, narcotic drugs such as morphine, synthetic drugs with narcotic properties such as tramadol, and various others. Some other classes of drugs not normally considered analgesics are used to treat neuropathic pain syndromes; these include tricyclic antidepressants and anticonvulsants. Analgesics are frequently used in combination, such as the paracetamol and codeine preparations found in many non-prescription pain relievers. They can also be found in combination with vasoconstrictor drugs such as pseudoephedrine for sinus-related preparations, or with antihistamine drugs for allergy sufferers. The use of paracetamol, as well as aspirin,

ibuprofen, naproxen, and other NSAIDS concurrently with weak to mid-range opiates (up to about the hydrocodone level) has been shown to have beneficial synergistic effects by combating pain at multiple sites of action. NSAIDs reduce inflammation which, in some cases, is the cause of the pain itself while opiates dull the perception of pain thus, in cases of mild to moderate pain caused in part by inflammation[1]. The plant-derived secondary metabolites have, over the years, greatly contributed to our current understanding of the important mechanisms related to the process of pain transmission and treatment. Furthermore, they have permitted us to characterize receptor types and identify endogenous ligands involved in the mechanism of nociception. In this review, we discuss the recent advances that have occurred regarding plant-derived substances in the process of development of new analgesic drugs. Plants, such as *Papaver somniferum*, *Cannabis sativa* and those of the Capsicum and Salix species, have greatly accounted for the development of clinically relevant drugs, which are useful for the management of pain disorders. The recent advances in our understanding of the mechanisms of action of the above plant-derived substances, together with use of molecular biology techniques, have greatly accelerated attempts to identify promising targets for the discovery of new, safe and efficient analgesic drugs. Despite the great progress that has occurred in the elucidation of pain transmission and despite decades of use, leaving aside its known undesirable side effects, morphine continues to be one of the most used drugs in clinical practice for the treatment of pain disorders. Thus, safer and more efficacious analgesic drugs are urgently needed. A search through the literature reveals that many potentially active antinociceptive plant-derived compounds have been identified. However, studies aiming to investigate their cellular and molecular mechanisms of action and well-controlled clinical trials to prove their efficacy in humans are still lacking. Nevertheless, natural or synthetic substances that bind to vanilloid or cannabinoid receptors, or even those that are capable of modulating the endogenous ligands which bind to these receptors, are expected soon to appear to assist in the treatment of several pain disorders, including those of neuropathic or neurogenic origin[2]. Regulation of body temperature requires a delicate balance between the production and loss of heat and the hypothalamus regulates the set point at which body temperature is maintained. In fever the set point is elevated and paracetamol like drugs promote its return to normal. These drugs do not influence body temperature when it is elevated by such factors as exercise or increase in

the ambient temperature[3]. Fever (also known as pyrexia, or a febrile response, and archaically known as ague) is a medical symptom that describes an increase in internal body temperature to levels that are above normal (37°C, 98.6°F). Fever should not be confused with hyperthermia, which is an increase in body temperature over the body's thermoregulatory set-point (normally approximately 37°C, but increased during a fever). A fever is most accurately characterized as a temporary elevation in the body's thermoregulatory set-point, which is usually by about 1-2°C. This elevation in thermoregulatory set-point means that the previous "normal body temperature" would be considered hypothermic. Effector mechanisms, such as increased blood pressure, increased heart rate, activation of brown adipose tissue and muscular shivering attempt to counteract the perceived hypothermia, thereby reaching the new thermoregulatory set-point. It is the most common symptom of many diseases. Most people take medication against fever because the symptoms cause discomfort. Fever increases heart rate and metabolism, thus potentially putting an additional strain on elderly patients, patients with heart disease, *etc*. This may even cause delirium. Therefore, potential benefits must be weighed against risks in these patients. In any case, fever must be brought under control in instances when fever escalates to hyperpyrexia and tissue damage is imminent. An adaptive mechanism, fever is the body's reaction to pathogens; it attempts to raise core body temperature to levels that will speed up the actions of the immune system, and may also directly denature, debilitate, or kill the pathogen. Most fevers are caused by infections, and almost all infectious diseases can cause fever. However, there are instances when fever escalates to temperatures where the body is at risk of destroying its own cells and must be brought under control with suppressive medication[4-13].

Antipyretic drugs are drugs that prevent or reduce fever by lowering the body temperature from a raised state. However, they will not affect the normal body temperature if one does not have fever. Antipyretics cause the hypothalamus to override an interleukin-induced increase in temperature. The body will then work to lower the temperature and the result is a reduction in fever[14]. Medicinal plants are an important source of new chemical substances with potential therapeutic effects. The research into plants with alleged folklore use as pain relievers should therefore be viewed as a fruitful and logical strategy in the search of new analgesic drugs. In addition, although there is a wide availability of clinically useful anti-inflammatory and analgesic drugs, a continuing

search for new effective agents with less unwanted side-effects remains vital. The screening of Natural products has led to the discovery of so many potent antipyretic drugs[15]. This present study was carried out to assess the validity of the folkloric uses of the two plants i.e. *K. rotunda* and *E. cannabinum* in the management of pain and treatment of fever and establish the possible mechanisms of pharmacological action.

9.2. Materials and methods

9.2.1. Plant materials

Methanol extracts of rhizomes of *K. rotunda* and leaves of *E. cannabinum* were subjected as test drug in these experiments.

9.2.2. Drugs and chemicals

All the chemicals used in this study were of analytical grade. Sodium CMC and methanol were obtained from LOBA chemicals, Kolkata. Pentazocine and paracetamol were obtained from Zydus-Cadila Ltd. Sikkim as a gift sample. Brewer's yeast (AR grade) was purchased from Sigma Chemicals. Methanolic extract of rhizomes of *K.rotunda* and leaves of *E. cannabinum* used in this study was extracted in our laboratory.

9.2.3. Test compound formulations

Oral suspensions of the leaves and rhizome extract were prepared by suspending them separately in 0.5% solution of sodium carboxy methylcellulose to obtain suitable dosage forms.

9.2.4. Experimental animals

Albino rats of Wistar strain (150-200 g) and Swiss albino mice (25-30 g) of either sex were procured from the central animal house of the Institute. They were housed in standard polypropylene cages and kept under controlled room temperature (24±2°C) and relative humidity (60-70%) in a 12h light-dark cycle. The animals were given a standard laboratory diet and water *ad libitum*. Food was withdrawn 12h before and during the experimental hours. All experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC).

9.3. Screening of Analgesic activity using Tail immersion method[16]

9.3.1. Analgesic activity of methanol extract of rhizome of *K. rotunda*

Tail immersion method was used to determine the analgesic activity. Swiss albino mice (25-30 g) were randomly divided into 4 groups having 6 animals each and they were fasted overnight but during the experiment had free access to water. Methanolic extract of the plant *K. rotunda* was administered orally (200 and 400mg/kg) 60 minutes prior to the commencement of the estimation of reaction time. Control group of mice (n = 6) received 0.5% w/v solution of sodium CMC in normal saline (0.1ml/100g), and the mean reaction time (in seconds) was determined. Test groups of mice were treated with the methanol extracts of rhizomes and leaves at doses 200 and 400mg/kg orally. Standard group of mice received 10mg/kg pentazocine. The temperature of water in the organ bath was set at 55±0.5°C with the help of a thermostat. Mice were held in position in a suitable restrainer with the tail extending out. The reaction time was determined by immersing the tail in hot water and the time taken by the mice to withdraw its tail clearly out of water was noted. Observations were repeated at an interval of 15 minutes up to 60 minutes.

9.3.2. Analgesic activity of methanol extract of leaves of *E. cannabinum*

The analgesic activity of methanol extract of *E. cannabinum* leaves was also determined as per the methods described above in 9.3.1 for *K. rotunda*.

9.4. Screening of Antipyretic activity using yeast-induced hyperpyrexia method[17]

9.4.1. Antipyretic activity of methanol extract of rhizome of *K. rotunda*

The antipyretic activity of methanolic extracts of rhizomes of *K. rotunda* was screened by using yeast-induced hyperpyrexia method. Briefly, pyrexia was induced in rats by injecting 10 ml/kg of 20% (w/v) suspension of Brewer's yeast intramuscularly. After 18h, the animals developed 0.5°C or more rise in the rectal temperature. They were distributed into 4 groups of 6 each and the extract at the doses of 200 and 400mg/kg was administered orally to the test groups (two groups). Standard group was administered with Paracetamol (33mg/kg) orally. Control group was given 0.5% w/v solution of sodium CMC in normal saline (0.1ml/100g). At different time intervals (1hr, 2hr and 3hr) rectal temperature was noted. Similarly, over night fasted normal animals were divided

into four different groups of 6 each and the experiment was carried out in the same manner as described above. Percentage reduction in rectal temperature was calculated by considering the total fall in temperature to normal level as 100%.

9.4.2. Antipyretic activity of methanol extract of leaves of *E. cannabinum*

The antipyretic activity of methanol extract of *E. cannabinum leaves* was determined as per the methods described above in 9.4.1 for *K. rotunda.*

9.5. Statistical methods

Statistical significance was analyzed using one way ANOVA. P value less than 0.05 was considered as significant.

9.6. Results

The effects of methanolic extracts of rhizomes of *K. rotunda* rhizomes and leaves of *E. cannabinum* on tail immersion method using mice are epitomized in table- 9.1 and table-9.2 respectively. The tail-immersion test is reported to be specific for agents producing central antinociceptive activity. Both the extracts show a good degree of analgesic activity at doses 200 and 400mg/kg in comparison with pentazocine (10mg/kg). The analgesic activity exhibited by the methanolic extracts of both the plants may be due to inhibitory effect on histamine, 5-HT and kinin like substance.

The outcomes of effects of methanolic extracts of rhizomes of *K. rotunda* and leaves of *E. cannabinum* on yeast-induced pyrexia in rats are depicted in table- 9.3 and table- 9.4 respectively. *K. rotunda* produced significant antipyretic effects $p<0.05$ in a dose dependent manner. A significant effect was observed at 200 and 400mg/kg dose of *K. rotunda* and *E. cannabinum* with compared to the standard drug paracetamol (33mg/kg).

Table 9.1. Effect of methanolic extract of rhizomes of *K. rotunda* on tail-immersion test in mice.

			Reaction time (sec) ± SEM			
Treatment	**Dose**	**Basal reaction time (sec) (X±SEM)**	**15min (X±SEM)**	**30min (X±SEM)**	**45min (X±SEM)**	**60 min (X±SEM)**
Control 0.5% w/v sodium CMC	0.1ml/100g	2.32±0.33	2.33±0.192	2.54±0.204	2.66±0.192	2.71±0.345
Pentazocine	10mg/kg	2.21±0.28	4.00±0.408	5.50±0.390	6.83±0.723	8.16±0.597
Plant extract	200mg/kg	2.66±0.24	2.50±0.204	2.83±0.281	3.17±0.366	3.33±0.192
Plant extract	400mg/kg	2.43±0.25	3.50±0.204	4.66±0.500	5.88±0.549	6.75±0.710
One way ANOVA F 4.278			All values are statistically significant respect to control at 5% level of significance			

Table 9.2. Effect of methanolic extract of leaves of *E.cannabinum* on tail-immersion test in mice.

			Reaction time (sec) ± SEM			
Treatment	**Dose**	**Basal reaction time (sec) (X±SEM)**	**15min (X±SEM)**	**30min (X±SEM)**	**45min (X±SEM)**	**60 min (X±SEM)**
Control 0.5% w/v sodium CMC	0.1ml/100g	2.40±0.20	2.49±0.28	2.57±0.19	2.68±0.20	2.73±0.19
Pentazocine	10mg/kg	2.33±0.192	4.167±0.37	5.50±0.311	7.0±0.235	8.50±0.204
Plant extract	200mg/kg	2.67±0.192	3.170±0.152	3.33±0.192	3.5±0.312	3.63±0.208
Plant extract	400mg/kg	2.50±0.204	3.50±0.204	4.66±0.385	6.66±0.451	8.33±0.304
One way ANOVA F 3.116			All values are statistically significant respect to control at 5% level of significance			

Table 9.3. Effect of methanolic extract of rhizomes of *K. rotunda* on Yeast induced pyrexia model in rats.

		Rectal temperature (°C)		Rectal temperature after administration of drug (°C)		
Treatment	**Dose (mg/kg)**	**(A) Normal animals (X±SEM)**	**(B) 18h after yeast administration (X±SEM)**	**(C1) 1h (X±SEM)**	**(C2) 2h (X±SEM)**	**(C3) 3h (X±SEM)**
Control (Normal saline)	0.5 ml	37.97±0.17	38.70±0.16	38.68±0.21 (2.73±3.21)	38.67±0.18 (4.10±4.06)	38.65±0.04 (6.84±7.01)
Paracetamol	33	37.72±0.14	38.40±0.12	38.05±0.27 (51.47±5.31)	37.95±0.21 (66.17±6.87)	37.80±0.14 (88.23±9.68)
Plant extract	200	37.47±0.17	38.45±0.20	38.15±0.17 (30.61±4.31)	38.02±0.32 (43.57±5.37)	37.97±0.22 (48.97±7.68)
Plant extract	400	37.43±0.20	38.42±0.19	38.10±0.16 (32.32±4.56)	37.96±0.17 (46.46±4.96)	37.87±0.20 (55.55±6.75)
One way ANOVA F 3.567		All values are statistically significant respect to control at 5% level of significance				

% reduction = B- Cn / B-A x 100, where n= 1, 2, 3 represented in the table in parenthesis.

All the values are mean±SEM (n=6)

Table 9.4. Effect of methanolic extract of leaves of *E. cannabinum* on Yeast induced pyrexia model in rats.

		Rectal temperature (°C)		Rectal temperature after administration of drug (°C)		
Treatment	**Dose (mg/kg)**	**(A) Normal animals (X±SEM)**	**(B) 18h after yeast administration (X±SEM)**	**(C1) 1h (X±SEM)**	**(C2) 2h (X±SEM)**	**(C3) 3h (X±SEM)**
Control (Normal saline)	0.5 ml	37.89±0.23	38.58±0.19	38.56±0.25 (2.89±2.98)	38.53±0.21 (7.24±8.21)	38.50±0.16 (11.59±12.21)
Paracetamol	33	37.78±0.24	38.51±0.21	38.15±0.26 (49.31±5.48)	38.08±0.16 (58.90±6.54)	37.89±0.18 (84.93±9.08)
Plant extract	200	37.56±0.28	38.55±0.29	38.21±0.23 (34.34±4.31)	38.07±0.27 (48.48±4.87)	37.93±0.14 (58.58±6.32)
Plant extract	400	37.54±0.23	38.52±0.22	38.13±0.24 (39.79±4.89)	38.00±0.19 (53.06±5.35)	37.87±0.26 (66.32±7.25)
One way ANOVA F 1.556		All values are statistically significant respect to control at 5% level of significance				

% reduction = B- Cn / B-A x 100 where n= 1, 2, 3 represented in the table in parenthesis

9.7. References

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