# CHAPTER - 8

# STUDIES ON ANTI-INFLAMMATORY ACTIVITY

## 8.1. Introduction

In traditional practice, medicinal plants are used to control inflammation in many countries. This has caused an increase in the number of experimental and clinical investigations directed towards the validation of the anti-inflammatory properties which are putatively attributed to these remedies[1,2]. The inflammatory mechanisms in the body are very complicated and they cannot be attributed to a single mediator or factor. Inflammation mediators, such as histamine, serotonin, arachidonic acid metabolites and quinines, are known to have a major role in generation of the inflammatory reactions[3,4]. Inflammatory process has two phases: acute and chronic. Acute inflammation is characterized by fever, pain, and edema, while chronic inflammation is characterized by cellular proliferation[5]. Complement system, fibrinolytic system and hyaluronidase enzyme are activated in plasma during inflammation[6]. Hyaluronidase activity in blood is increased during inflammation and the decrease in inflammation parallels a decrease in hyaluronidase activity[7]. Models of acute inflammation, which induce inflammation by administration of formalin, dextran, histamine, serotonin, bradykinin, prostaglandin and carrageenan are used to investigate anti-inflammatory effects of drugs[8]. Carrageenan-induced inflammation model is a COX-dependent reaction and is used to determine COX inhibition[9]. Prolonged uses of both steroidal and non-steroidal anti-inflammatory drugs are well known to be associated with peptic ulcer formation[10]. Hence, search for new anti-inflammatory agents that retain therapeutic efficacy and yet are devoid of these adverse effects is justified. There is much hope of finding active anti-inflammatory compounds from indigenous plants as these are still used in therapeutics despite the progress in conventional chemistry and pharmacology in producing effective drugs. Herbal drugs are being proved as effective as synthetic drugs with lesser side effects. The enzyme, phospholipase $A_2$, is known to be responsible for the formation of mediators of inflammation such as prostaglandins and leukotrienes which by attracting poly-morphonuclear leucocytes to the site of inflammation would lead to tissue damage probably by the release of free radicals. Phospholipase $A_2$ converts phospholipids in the cell membrane into arachidonic acid, which is highly reactive and is rapidly metabolized by cycloxygenase (prostaglandin synthase) to prostaglandins, which are major components that induce pain and inflammation[11,12].

Rheumatoid arthritis is a highly variable, chronic inflammatory condition affecting mostly diarthrodial (hinge-like) joints but often with articular and systemic involvement. The available data indicate that 76% of patients with rheumatoid arthritis are taking NSAIDs[13,14]. Apart from treating the underlying disease, it is necessary to relieve patient's pain. This has led to major improvements in the treatment of acute and chronic pain. In the pharmacological treatment of acute pain, aspirin-like and morphine-like drugs still form the cornerstone of most therapies[15]. There was a controversy about the anti-inflammatory effect of *Harpagophytum procumbens* (devil's claw), an herbal product marketed in Canada and Europe, as a home remedy for relief of arthritic diseases.

Recent studies suggest that *Harpagophytum procumbens* has anti-inflammatory and analgesic effect. Extract of *Harpagophytum procumbens* have become the focus of research as a potential therapeutic agent in the treatment of rheumatic arthritis and pain due to its favorable side effects profile compared to synthetic alternatives[16]. *Harpagophytum procumbens* was effective in the treatment of osteoarthritis and reduced the need for analgesic and NSAIDs therapy[17]. Treatment of 800 mg of the extract, three times daily with total dose of not more than 2400mg per day has been accompanied by reduction of pain[18]. *Harpagophytum procumbens* can probably help many of those who suffer from low back pain with fewer side effects than NSAIDs treatment that are troublesome in the elderly, at a cost that is certainly not excessive[19]. In Indian system of medicine, a large number of drugs of either herbal or mineral origin have been advocated for various types of diseases and other different unwanted conditions in humans[20]. Ayurveda is one of the traditional systems of medicine practiced in India and Sri Lanka and can be traced back to 6000 BC[21]. Ayurvedic medicines are largely based upon herbal and herbomineral preparations and have specific diagnostic and therapeutic principles [22]. Inflammation is a disorder involving localized increases in the number of leukocytes and a variety of complex mediator molecules[23]. Prostaglandins are ubiquitous substances that indicate and modulate cell and tissue responses involved in inflammation. Their biosynthesis has also been implicated in the pathophysiology of cardiovascular diseases, cancer, colonic adenomas and Alzheimer's disease[24,25].

Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects[26,27]. The research into plants with alleged folkloric use, as

pain relievers, anti-inflammatory agents, should therefore be viewed as a fruitful and logical research strategy in the search for new analgesic and anti-inflammatory drugs[28]. Scientific interest in medicinal plants has burgeoned in recent times due to increased efficiency of new plant derived drugs and rising concerns about the side effects of conventional medicine. Inflammation is seen in conditions such as Alzheimer's disease, cancer, irritable bowel syndrome and hepatic diseases. It is believed that controlling inflammation may help to alleviate these conditions or even prevent them[29]. An attempt was made in this present study to assess the efficacy of two indigenous herbs for their anti-inflammatory activity in rats. Thus the present investigation was carried out to evaluate the anti-inflammatory potential of *K. rotunda* and *E. cannabinum.*

## 8.2. Materials and methods

### 8.2.1. Plant materials

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

### 8.2.2. Drugs and chemicals

Carrageenan and Indomethacin was obtained from Sigma-Aldrich, Germany. All the solvents used were of analytical grade procured from E. Merck, Mumbai.

### 8.2.3. Experimental animals

Albino rats of Wistar strain (150-200g) 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$^0$C); relative humidity 60-70%) in a 12h light-dark cycle. The rats 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).

## 8.3. Screening of Anti-inflammatory activity by using Carrageenan Induced Rat Paw Edema

### 8.3.1. Anti-inflammatory activity of methanol extract of rhizome of *K. rotunda*

Anti-inflammatory activity was assessed by the method described by Winter *et. al.*[30]. The rats were divided into four groups of six animals each. First group (negative control) received 0.2ml/100g b.w. of 0.5% w/v solution of sodium CMC in normal saline, second group (positive control) received 10mg/kg p.o. indomethacin. The third and fourth group received methanolic extract (200, 400mg/kg p.o.) of *K. rotunda* respectively. After 1h, the rats were challenged with subcutaneous injection of 0.1ml of 1% w/v solution of carrageenan into the plantar side of the left hind paw. The paw was marked with ink at the level of lateral malleolus and immersed in mercury cup up to the mark. The plethysmograph apparatus used for the measurement of rat paw volume was that of Singh and Ghosh[31]. The paw volume was measured immediately after injection (0h) and then every hour till 3h after injection of carrageenan to each group. The difference between the initial and subsequent reading gave the actual edema volume. Percent inhibition of inflammation was calculated using the formula,

*% inhibition = 100 (1-Vt/Vc)*

Where 'Vc' represents edema volume in control and 'Vt' edema volume in groups treated with test extracts.

### 8.3.2. Anti-inflammatory activity of methanol extract of leaves of *E. cannabinum*

The anti-inflammatory activity of methanol extract of *E. cannabinum* leaves was determined as per the methods described above in 8.3.1 for *K. rotunda*.

## 8.4. Statistical analysis

All the experimental data were expressed as mean±SEM. The statistical significance of the differences was accessed with one way ANOVA. Differences with P values less than 0.05 was considered as significant.

## 8.5. Results

The anti-inflammatory effect of *K. rotunda* is represented in table-8.1 and the effect of *E. cannabinum* is shown in table-8.2. The methanolic extract of both *K. rotunda* and *E. cannabinum* exhibited significant ($p<0.05$) anti-inflammatory activity at doses of 200 and 400mg/kg body weight orally. As shown in table-8.1 and table-8.2 methanolic extract of *K. rotunda* and methanolic extract of *E. cannabinum* exhibited inhibition in rat paw oedema by 21.36% and 18.50% for 200mg/kg body weight and 50.97% and 47.56% for 400mg/kg body weight respectively whereas standard drug indomethacin showed 53.77% and 50.62% respectively.

The present study indicates the potential of these herbal drugs as anti-inflammatory drugs. Such drugs can successfully be used in various inflammatory diseases. The activity may be attributed to the inhibition of the COX-2 enzyme or inhibition of the activation of transcription factors. It can be concluded that all the extracts have potential to act as anti-inflammatory agents due to presence of flavonoids as active constituent. It can be concluded that both the extracts have potential to be explored as anti-inflammatory agents.

**Table-8.1. The effect of methanolic extract of *K. rotunda* on Carrageenan induced edema in rats**

| | | Volume of mercury displaced in ml. (± SEM) | | | | |
|---|---|---|---|---|---|---|
| **Treatment** | **Dose** | **0hr (X±SEM)** | **1hr (X±SEM)** | **2hr (X±SEM)** | **3hr (X±SEM)** | **% inhibition of paw of edema** |
| Control 0.5% w/v sodium CMC | 0.2ml/100g | 0.996±0.048 | 1.297±0.032 | 1.607±0.029 | 1.712±0.036 | - |
| Indomethacin | 10mg/kg | 0.990±0.038 | 1.214±0.021 | 1.463±0.024 | 1.321±0.028 | 53.77 |
| Plant extract | 200mg/kg | 0.997±0.037 | 1.262±0.017 | 1.523±0.011 | 1.560±0.031 | 21.36 |
| Plant extract | 400mg/kg | 0.993±0.026 | 1.238±0.023 | 1.489±0.041 | 1.344±0.036 | 50.97 |
| One way ANOVA F 0.315 | | All values are statistically significant respect to control at 5% level of significance | | | | |

**Table-8.2. The effect of methanolic extract of *E. canabinum* on Carrageenan - induced edema in rats**

| | | Volume of mercury displaced in ml. (± SEM) | | | | |
|---|---|---|---|---|---|---|
| **Treatment** | **Dose** | **0hr (X±SEM)** | **1hr (X±SEM)** | **2hr (X±SEM)** | **3hr (X±SEM)** | **% inhibition of paw of edema** |
| Control (0.5% w/v sodium CMC) | 0.2ml/100g | 0.988±0.029 | 1.292±0.034 | 1.611±0.031 | 1.707±0.036 | - |
| Indomethacin | 10mg/kg | 0.979±0.031 | 1.213±0.024 | 1.448±0.028 | 1.334±0.020 | 50.62 |
| Plant extract 200mg/kg | 200mg/kg | 0.986±0.019 | 1.274±0.019 | 1.493±0.027 | 1.572±0.029 | 18.50 |
| Plant extract 400mg/kg | 400mg/kg | 0.984±0.036 | 1.241±0.041 | 1.483±0.036 | 1.361±0.040 | 47.56 |
| One way ANOVA F 0.238 | | All values are statistically significant respect to control at 5% level of significance | | | | |

## 8.6. References


1. Giron LM, Freire V, Alonzo A and Caceres A. Botanical survey of medicinal flora used by the Caribs. *J. Ethnopharmacol.*, 1991, **28**, 1956-1961.
2. Kumar VL and Basu N. Anti-inflammatory activity of the latex of *Calotropis procera*. *J. Ethnopharmacol.*, 1994, **44**, 123-125.
3. Maling HM, Webster ME, Williams MA, Saul W and Anderson W. Inflammation induced by histamine, serotonin, bradykinin and comp 48/80 in the rat: Aantagonists and mechanism of action. *J. Pharmacol. Exp. Ther.*, 1974, **191**, 300-310.
4. Portanova JP, Zhang Y, Anderson GD, Hauser SD, Masferrer JL, Seibert K and Gregory SA. Selective neutralization of prostaglandin E blocks inflammation, hyperalgesia and interleukin-6 production *in vivo*. *J. Exp. Med.*, 1996, **184**, 883-891.
5. Dunne MW. *Inflammation and repair.* In: Pathophysiology. Porth CM, (Ed), Lippincott, Philadelphia, 1990, p 165-176.
6. Collins T. *Acute and chronic inflammation.* In: Robbins Pathology Basic of Disease, Cortan RS, Kumar V and Collins T, (Eds), 6th edn., Saunders, Elsevier India, 2003, p 50-88.
7. Procida C, Montovani V and Bianchini P. Anti-hyaluronidase activity of some pyrazole derivatives. *Boll. Soc. Ital. Biol. Sper.*, 1971, **47**, 159-163.
8. Suleyman H and Buyukokuroolu ME. The effects of newly synthesized pyrazole derivatives on formaldehyde-carrageenan and dextran-induced acute paw edema in rats. *Biol. Pharm. Bull.*, 2001, **24**, 1133-1136.
9. Gamache DA, Povlishock JT and Ellis EF. Carrageenan induced brain inflammation and characterization of the model. *J. Neurosurg.*, 1986, **65**, 675-685.
10. Ewart A. *Remington's Pharmaceutical Sciences.* eds., Gennaro AF, (Ed), 16th edn., Mack Publishing Company, Easton, Pensyllvenia, 1980, p 873-874.
11. Higgs GA, Moncada S and Vane JR. Eicosanoids in inflammation. *Ann. Clin. Res.*, 1984, **16**, 287-99.

12. Vane JR. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nat. New. Biol.*, 1971, **231**, 232-235.

13. Lanhers *et. al.* How recent findings affect thinking about nonsteroidal anti-inflammatory drugs? *Drugs*, 1997, **53(4)**, 563-582.

14. Schiff M. Emerging treatments for rheumatoid arthritis. *Am. J. Med.*, 1997, **102(1)**, 11-15.

15. Besson JM. The neurobiology of pain. *Lancet*, 1999, **353**, 1610-1615.

16. Fiebich BL, Kammerer N, Heinrich M and Hiller O. Inhibition of TNF-alpha synthesis LPS-stimulated primary human monocytes by Harpagophytum extract Stei Hap 69. *Phytomedicin*, 2001, **8(1)**, 28-30.

17. Leblan D, Chantre P and Fournie B. *Harpagophytum procumbens* in the treatment of knee and hip osteo-arthritis. Four-month results of a prospective, multicenter, double-blind trial versus diacerhein. *Joint Bone Spine.*, 2000, **67(5)**, 462-467.

18. Chrubasik S, Zimpfer Ch, Schutz U and Ziegler R. Effectiveness of *Harpagophytum procumbens* in treatment of acute low back pain. *Phytomedicine*, 1996, **3(1)**, 1-10.

19. Chrubasik S, Junck H, Breitschwerdt H, Conradt C and Zappe H. Effectiveness of *Harpagophytum* extract in the treatment of exacerbation of low back pain: A randomized, placebo controlled, double-blined study. *Eur. J. Anaesthesiology*, 1999, **16**, 118-129.

20. Brekhman C and Dardimov I. New substances of plant origin, which increase non-specific resistance. *Annu. Rev. Pharmacol.*, 1969, **21**, 219-226.

21. Charak Samhita (Trans): Shree Gulab Kunverba Ayurvedic Society, Jamnagar, India, 1949.

22. Patwardhan B and Hopper B. Ayurvedic and future drug development. *J. Alter. Coplement Med.*, 1992, **19(12)**, 9-10.

23. Mantri P and Witiak DT. Inhibition of cyclooxygenase and 5-lipoxygenase. *Curr. Med. Chem.*, 1994, **1**, 328-355.

24. Smith WL and De Witt DL. Biochemistry of prostaglandins endoperoxide H synthase-1 and synthase-2 and their differential susceptibility to NSAIDs. *Sem. Nephrol.*, 1995, **15**, 179-194.

25. Lipsky P. The clinical potential of COX-2 specific inhibitors. *Amer. J. Med.*, 1999, **106**, 51-57.

26. Farnsworth NR. *Screening plants for new medicines*. In: Biodiversity, Part II, Wilson EO, (Ed), National Academy Press, Washington, 1989, p 83-97.

27. Eisner T. *Chemical prospecting: A call for action*. In: Ecology, Economic and Ethics, Borman FH and Kellert SR, (Eds), The Broken Circle, Yale University Press, New Haven, 1990, p 258.

28. Elisabetsky E, Amodor TA, Albuquerque RR, Nunes DS and Carvalho ACT. Analgesic activity of *Psychotria colorata* (Wildex R. & S.) Muell Arg. Alkaloids. *J. Ethnopharmacol.*, 1995, **48**, 77-83.

29. Omkar TJ and Gadgoli C. Evaluation of Anti-inflammatory activity of *Nyctanthes arbor-tristis* and *Onosma echioides*. *Pharmacognosy Magazine*, 2006, **2(8)**, 258-60.

30. Winter CA, Risley EA and Silber RH. Anti-inflammatory activity of indomethacin and plasma corticosterone in rats. *J. Pharmacol. Exp. Ther.*, 1968, **162**, 196-201.

31. Singh H and Ghosh MN. Modified plethysmometer for measuring foot volume of unanesthetized rats. *J. Pharm. Pharmacol.*, 1968, **20**, 316-317.