Chapter 5

Antiinflammatory and analgesic activity of *Swertia* spp. found in Kashmir valley

5.1. Introduction

Inflammation is a complicated biological phenomenon, which in short can be defined as reaction of tissues to injury in higher animals. The pathophysiological study of the disease shows complex vascular, lymphatic and local tissue reactions at the site of injury. Essentially, it is a normal and necessary defense and repair response of the body. The basic symptomatic features of inflammation are swelling, heat pain, redness and subsequent reduction in structural and functional organization (Vane and Ferreira, 1978). The basic difference between acute and chronic inflammation lies in the duration of inflammatory process. Acute inflammation lasts for a week or two, whereas as chronic inflammation lasts for months, sometimes years or whole life and is associated with increased activity of cellular components. Inflammation is characterized by three phases, which are interrelated and merged one into another. These are:

5.1.1: Transudative phase

It is the first phase of inflammatory response, also known as degenerative stage. In this phase cellular degradation occurs in the traumatized area. The vessels are destroyed and haemorrhage ensues which leads to localized compression and necrosis. The epithelial cells and some of the fibroblasts as well as their cytoplasm become vacuolated and their nuclei undergo pyknosis and karyorrhexis. Various highly active substances are liberated at the site of injury, which include histamine, serotonin, kinins, leucotoxin exudin (Kallermeyer and Graham, 1968), prostaglandins (Vane and Ferreira, 1978), thromboxane A₂ (Hamberg et al. 1976), prostacyclin (Moncada and Vane, 1991), besides some other mediators of inflammation. These substances also called as chemical mediators cause dilatation of blood vessels and increase their permeability leading to erythema and oedema at the site of injury. Various proteolytic enzymes are released in high concentration at the site of injury and act as mediator genase for production
of polypeptides as bradykinin and kallidin (Withelm, 1971). There are number of evidences to suggest that not only endogenous proteases participate in the development of inflammatory reactions but also exogenous enzymes can act as inflammatogenic stimulus (Fisher, 1974). In the inflammatory process prostaglandins are released at concentration that indicates their potential role in the inflammatory reaction (Flower, 1974). These induce most of the signs of inflammation, erythema, pain, vasodilatation and oedema. The fatty acid hydroperoxides have been shown to cause erythema and pain (Ferreira, 1972). Platelet aggregation is believed to play an important role in the initiation of micro-thrombi and vascular inflammation (Mustard and Packham, 1970). Platelets are the storage pools of adenine dinucleotide (ADP), serotonin (5-HT), histamine, prostaglandins, calcium ions and α-granules. These permeability factors are released due to aggregation and damage of platelets induced by ADP, adrenaline and thrombi.

5.1.2: Exudative phase

The passive phenomenon of haemorrhage and necrosis are followed by active tissue and cellular reactions such as vascular dilation and diapedesis giving rise to leucocyte migration. There is increase in capillary number engorging with blood or haemostasis. This alteration is responsible for heat and redness observed in inflamed areas. Active congestion develops at the edge of the wound followed by extravasation of serous fluid from the lumen of the dilated vessels in the extravascular spaces, which soon accompanies the vasodilatation giving rise to local oedema. (Spector, 1969). A number of lytic enzymes including lysosomal enzymes and hydrolases are released into the inflammatory exudates either from injured local cells or during phagocytic activity of migrating cells (Weissmann et al, 1971). These enzymes such as acid phosphatase, β-glucuronidase, hyaluronidase and phospholipases cause further damage of the injured tissue by lysing them and the fluids originating from the necrotic process mixed with the serous material that extruded from the vessels, yield characteristic material called pus.

5.1.3: Proliferative phase

The repair or Proliferative phase is the last phase of inflammatory reaction. It includes proliferation of capillaries and young fibroblasts. The epithelium of young fibroblasts start elaborating collagen fibres. The cellular proliferation penetrates the exudates producing a highly

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vascularized reddish mass termed "granulation tissue", which develops to fill the wedge of wound. A number of enzymes such as cathapsin, lipases and nucleases catalyze the degradation and cleaning of inflammatory debris.

In recent times, remedies are being looked into the nature itself. In the past decade, there has been a tremendous increase in the search for newer drugs of plant origin with a hope for finding the ideal remedies for some crippling inflammatory diseases.

Many plants are being tried as anti-inflammatory agents. Swertia chirata is widely used in India for various ailments. It has also shown anti-inflammatory activity (Nadkarni et al., 1982; Islam et al., 1985; Mandal et al., 1992).

Inhibition of carrageenan-induced inflammation is one of the most suitable test procedures to screen anti-inflammatory agents. The development of carrageenan-induced oedema is biphasic, the first phase is attributed to the release of histamine, 5-HT, and kinins, while the second phase is related to the release of prostaglandins (Larson et al., 1983; Brooks et al., 1991; Vane et al., 1987).

Despite the availability of many allopathic medicine (steroidal and nonsteroidal anti-inflammatory drugs) for the treatment of chronic inflammation and/or acute pain, research is on for finding newer anti-inflammatory agents that affect only the aberrant, uncontrolled inflammation by modifying inflammatory response without interfering the normal inflammatory process, which is an essential part of body’s vital defense mechanism to its major environmental insults or invading microorganisms. One of the Swertia species that is well recognized for the anti-inflammatory action in animal model is S. chirata. This species is widely used in India for various ailments, and its anti-inflammatory activity has been published in literature (Nadkarni et al., 1982; Islam et al., 1985; Mandal et al., 1992). The phytochemical constituents responsible for the anti-inflammatory action of Swertia chirata are dimethylxanthane, mangiferin, α-mangostin, α-mangostin triacetate and isomangostin. Structures of some of these bioactive chemical constituents are shown in Figure 5.1.

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Swertia species found in the Kashmir valley, *Swertia petiolata* and *Swertia tetragona* have not been investigated, in spite of the larger probability of these species being anti-inflammatory. The following text would highlight the results of our study on the anti-inflammatory activity of the *Swertia* species of the Kashmir valley, and would provide an insight into the possible mechanism of action underlying the pharmacological effect.

### 5.2. Experimental Design

#### 5.2.1 Preparation of extracts

Shade dried rhizomes and the whole plant of *S. petiolata* and *S. tetragona* were crushed in a mixer grinder. The crushed materials were subjected to extraction in soxhlet apparatus at 60-70°C for 6 hours continuously in hydro-methanol (20:80). The extracted materials were evaporated to dryness under reduced pressure at 40-50°C. The crude extracts were dried and suspended in distilled water and used in further studies. Percent yield of the extract was as follows: *S. petiolata* 20.3 %, and *S. tetragona* 11.2%. Similarly, both the herbs were cold extracted with water for 24 hours with occasional stirring. The extracts were filtered and dried and the percent yield calculated as 15.5% and 10.2% for *S. petiolata* and *S. tetragona* respectively.

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5.2.2. Evaluation of the antinflammatory activity

As described in the methods section, rats were divided into groups as follows: Group I (Normal Control) received normal saline, Group II rats (Inflammation model) were injected carrageenan, while Group III, IV, V and VI rats (Experimental rats) were treated with carrageenan and the *Swertia* extracts. Group VII rats were injected with carrageenan and treated with Aceclofenac (5 mg/kg body weight) as a standard allopathic antinflammatory drug. The doses of the extracts administered to rats were 1gm/kg body weight for aqueous extracts and 200 mg/ kg for hydro-alcoholic extracts. The measurement of hind paw volume was carried out1,2 and 3 hours after carrageenan injection using plethysmograph. Detailed procedure for the induction of inflammation in rats is described in chapter-3.

5.2.3. Analgesic Activity

Male rats (Wistar strain) were divided into groups as above. In addition to the control rats, experimental rats from Group II and III received, respectively, the aqueous and the hydro-alcoholic extracts of *S. petiolata*. Group IV and V received the aqueous and the hydro-alcoholic extracts of *S. tetragona*, and Group VII received Aceclofenac (5 mg/kg body weight). Analgesic activity of each extract was determined using standard pharmacological methods (tail flick method, hot plate method, and tail clip method), as described in detail in the methods section (Chapter 3).

5.3. Results

5.3.1. Antiinflammatory studies

Carrageenan is widely used to demonstrate the anti-inflammatory activity of the extracts prepared from the medicinal plants, and is considered a satisfactory method for such studies. Antiinflammatory effect of the two *Swertia* species against carrageenin-induced inflammation is shown in Figure 5.1. The herbs significantly reduced the paw volume as compared to the control rats. The degree of reduction of paw volume was comparable with the standard antiinflammatory drug Aceclofenac.
A comparison of the mean percentage increase in hind paw volume due to carrageenan-induced edema in saline treated control and extract treated rats was used to calculate the percent anti-inflammatory activity of the aqueous extracts of *S. petiolata* and *S. tetragona*. Inflammation observed in saline treated control animals was taken as 100%. The hind paw volume was noted at 1, 2 and 3 hours post treatment. Hydro-alcoholic extract of *S. petiolata* decreased the inflammation by 66.66%, 68.10% and 77.7% at 1, 2 and 3 hours post treatment, while as the aqueous extract decreased the inflammation by 68%, 68% and 70.30% respectively. In the same manner the hydro-alcoholic extract of *S. tetragona* decreased the inflammation by 70%, 72.10% and 74.07%, respectively, while in similar experimental conditions the aqueous extract decreased the paw volume by 56%, 59% and 66.66%. Aceclofenac (used as a standard anti-inflammatory allopathic control) could alleviate the inflammation to 62%, 64.05% and 66.66% under the identical experimental conditions.

### 5.3.2. Analgesic studies

Analgesic activity of the extracts was determined by tail flick method 3 hrs after the extract treatment. All the extracts showed good analgesic activity (Figure 5.2), as evidenced by an increase in the reaction time by 8 and 7.5 seconds in case of *S. petiolata* (hydro-alcoholic and aqueous extracts, respectively), and 7.5 and 8.5 seconds in case of *S. tetragona* (hydro-alcoholic and aqueous extracts, respectively). Aceclofenac under the similar conditions increased the reaction time by 9.5 seconds. The analgesic activity was also confirmed by tail clip and hot plate methods. In the tail clip method the increase in reaction time was by 8 and 7.7 seconds in case of *Swertia petiolata* (hydro-alcoholic and aqueous extracts) and 9 and 8.5 in case of *Swertia tetragona* (hydro-alcoholic and aqueous extracts). Under the similar experimental conditions, Aceclofenac increased the reaction time by 9.5 seconds. Similarly the reaction time in hot plate method was 8.5 and 7.5 in case of *Swertia petiolata* (hydro-alcoholic and aqueous extracts) and 7.7 and 7.5 in case of *Swertia tetragona* (hydro-alcoholic and aqueous extracts). Under the identical experimental conditions, Aceclofenac increased the reaction time by 8 seconds.
Anti-inflammatory activity of the Swertia extracts  
(percentage reduction of paw volume)

Figure 5.1: Antiinflammatory activity of swertia extracts at different time intervals. S.P (H/A): *S. petiolata* hydro-alcoholic extract; S.P (Aq): *S. petiolata* aqueous extract; S.T (H/A): *S. tetragona* hydro alcoholic extract; and S.T (Aq): *S. tetragona* aqueous extract. Results are expressed as mean of 6 animals.
Analgesic activity of the swertia extracts
3 hours post treatment
(Increase in the reaction time in seconds)

![Graph showing analgesic activity of swertia extracts after 3 hours treatment.]

Figure 5.2: Analgesic activity of swertia extracts by different methods after 3hrs of treatment. S.P (H/A): S. petiolata hydro-alcoholic extract; S.P (Aq): S. petiolata aqueous extract; S.T (H/A): S. tetragona hydro alcoholic extract; and S.T (Aq): S. tetragona aqueous extract. Results are expressed as mean of 6 animals.
5.4. Discussion

Results presented in the above tables provide clear evidence suggesting the anti-inflammatory and analgesic role of *Swertia* species collected from the Kasmhir valley. Significant alleviation of pain and inflammation was demonstrated by the two species (Figure 5.1 and 5.2), which was comparable to aceclofenac, the standard anti-inflammatory and analgesic drug. The findings are in accordance with the earlier studies on other *Swertia* species, particularly *Swertia chirata*, which is widely used in India to treat fever, inflammatory diseases and malaria (Nadkarni, 1982; Islam et al., 1995; Mandal et al., 1992; Das et al., 2000). Further, those species have been found to possess antioxidant (Patro et al., 2004), antihepatotoxic (Reen et al., 2001; Lee et al., 2005; Hase et al., 1997; Mukherjee et al., 1997; Hajimehdipur et al., 2006), anticarcinogenic (Saha et al., 2004), and anthelmintic property (Iqbal et al., 2006).

Inhibition of carrageenan-induced inflammation is one of the most suitable test procedures to screen antiinflammatory agents. Oedema formation in paw is the result of a synergism between various inflammatory mediators that increase vascular permeability and/or mediators that increase blood flow (Ialenti et al., 1995). The development of carrageenan-induced oedema is bi-phasic: the first phase is attributed to the release of histamine, 5-hydroxytryptamine and kinins, and the second phase is related to the release of prostaglandins (Larson and Henson, 1983; Brooks et al., 1991; Vane and Booting, 1887). Both steroids and non-steroidal anti-inflammatory drugs (like aspirin and indomethacin) inhibit biosynthesis of prostaglandins. Steroids inhibit conversion of membrane phospholipids to arachidonic acid and thus blocks formation of leukotriene, prostacyclin, PGE2 and related prostaglandins whereas non-steroidal antiinflammatory drugs irreversibly inactivate cycle-oxygenase. Moreover, so far very few antiinflammatory agents have been shown to have direct antaqqistic action against exogenous prostaglandins. Oxygen derived free radicals and oxidants have been shown to play an important role in various forms of inflammation (Ialenti et al., 1995; Salvemini et al., 1996). In order to identify the compounds responsible for the biological activities observed, many compounds as Xanthones, mangiferin, α-mangostin, isomangostin, α-mangostin triacetate, besides some other compounds, as ursolic acid have been separated from the extracts of *S. chirata*, and reported to be antiinflammatory (Mandal et al., 1992; Banerjee et al., 2000).
Several reports are there which suggest a strong role of ursolic acid in antiinflammatory action. It not only inhibits human leucocyte elastase, but also 5-lipoxigenase and cyclooxygenase activity (Najid et al., 1992; Safayhi et al., 1997). The mechanisms of antiinflammatory activity of ursolic acid have been attributed to inhibition of histamine release from mast cells (Dai et al., 1989; Rajasekeran et al., 1990; Balanehru and Nagarajan, 1994) and to inhibition of complement activity (Dai et al., 1989; Kapil and Sharma, 1994). Moreover, ursolic acid exhibited strong inhibitory activity on the production of nitric oxide in macrophages (Ryu et al., 2000). Polyphenols were demonstrated as possessing in vivo antiinflammatory properties too (Moreira et al., 2000; Ueda et al., 2002).

Moreover, the irridoids and seco-irridoids could also contribute to antiinflammatory effect (De Mirando et al., 2000; Diaz et al., 2000). Flavonoids also have been known to possess antiinflammatory, antioxidant, antiallergic, hepatoprotective, antithrombotic, antiviral and anticarcinogenetic activities. To conclude it can be assumed that either the antiinflammatory and analgesic activity of both the herbs under investigation is due to one of these compounds or all of them may be acting synergistically at different levels of inflammation. This also gathers support from the fact that hydro-alcoholic extracts of both the herbs showed more antiinflammatory activity than their respective aqueous extracts, which may be due to the presence of more antiinflammatory phytoconstituents in hydro-alcoholic extracts than in aqueous extracts.

5.5. Conclusion

Herbs are an integral part of nature and contain various natural substances that can promote health. Both forms (aqueous and hydro-alcoholic) of the extracts have shown significant antiinflammatory activity with carrageenan induced paw oedema model in rats. The hydro-alcoholic extracts are found to possess greater antiinflammatory activity than aqueous extracts, which may be due to presence of more antiinflammatory principles in the hydro-alcoholic extract. In our study, the analgesic activity of these species highlighted the importance of the extracts in traditional preparations.