CHAPTER – 8

ANTI-INFLAMMATORY ACTIVITY OF COMBINED EXTRACT OF CISSUS QUADRANGULARIS & AEGLE MARMELOS

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8.1. Introduction

Inflammation is a composite response to injurious agents, like microorganisms and spoiled necrotic cells that consist of vascular responses, movement and activation of leucocytes, and systemic reactions. Inflammation is a local response of living mammalian tissues to injury due to any agent. It is a protective and defensive mechanism of body. They are two types acute and chronic. There are various components responsible to an inflammatory reaction such as edema formation, leukocyte infiltration and granuloma formation that can contribute to the associated symptoms and tissue injury. The classic inflammatory responses are calor (warmth), dolor (pain), rubor (redness) and tumor (swelling)\(^1\). Some of the inflammatory disorders include Atherosclerosis, asthma, irritable bowel syndromes, hepatitis, arthritis, colitis, nephritis etc.

Inflammatory responses occur in three different temporal phases and arbitrated by three mechanisms, (1) an acute phase, characterized by transient local vasodilation and increased capillary permeability (2) a delayed, subacute phase characterized by infiltration of leukocytes and phagocytic cells and (3) a chronic proliferative phase, in which tissue degeneration and fibrosis occur.

Number of mechanisms is occupied in the promotion and resolution of the inflammatory process\(^2\). Although earlier studies emphasized the promotion of migration of cells out of the microvasculature, recent work has focused on adhesive interactions, including the E-selectins, P-selectins and L-selectins, intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and leukocyte integrins, in the adhesion of
leukocytes and platelets to endothelium at places of inflammation. Activated endothelial cells play a key role in "targeting" circulating cells to inflammatory sites. Expression of the adhesion molecules varies among cell types involved in the inflammatory response.

Cell adhesion occurs by recognition of cell-surface glycoproteins and carbohydrates on circulating cells due to the augmented expression of adhesion molecules on resident cells. Thus, endothelial activation results in leukocyte adhesion as the leukocytes recognize newly expressed L-selectin and P-selectin; other important interactions include those of endothelial-expressed E-selectin with sialylated Lewis X and other glycoproteins on the leukocyte surface and endothelial ICAM-1 with leukocyte integrins. Novel classes of antiinflammatory drugs directed against cell-adhesion molecules are under active development but have not yet entered the clinical arena.

Histamine was one of the first identified mediators of the inflammatory process. Although several H\textsubscript{1} histamine–receptor antagonists are available, they are useful only for the treatment of vascular events in the early transient phase of inflammation. Bradykinin and 5-hydroxytryptamine (serotonin, 5-HT) also may play a role in mediating inflammation, but their antagonists improve 5fluoro uracic only some types of inflammatory response. Leukotriene (LT) receptor antagonists (montelukast and zafirlukast) exert anti-inflammatory actions and have been approved for the treatment of asthma.

Another lipid autacoid, platelet-activating factor (PAF), has been implicated as an important mediator of inflammation; however, inhibitors of PAF synthesis and PAF-receptor antagonists have proven disappointing in the treatment of inflammation.

8.1.1. Non steroidal Antiinflammatory Drugs (NSAIDs)

NSAIDs are a chemically heterogeneous group of compounds, often chemically unrelated (although most of them are organic acids), which nevertheless share certain therapeutic actions and adverse effects. Most currently available traditional NSAIDs act by
inhibiting the prostaglandin G/H synthase enzymes, colloquially known as the cyclooxygenases. The inhibition of cyclooxygenase-2 (COX-2) is thought to mediate, in large part, the antipyretic, analgesic and anti-inflammatory actions of traditional NSAIDs, while the simultaneous inhibition of cyclooxygenase-1 (COX-1) largely, but not exclusively, accounts for unwanted adverse effects in the gastrointestinal tract.

NSAIDs like Acetyl salicylic acid, irretrievably acetylates COX, along with several structural subclasses of traditional NSAIDs, including propionic acid derivatives, acetic acid derivatives and enolic acids, all of which battle in a reversible manner with the arachidonic acid (AA) substrate at the place of COX-1 and COX-2. Aspirin inhibits the COX enzymes but in a manner molecularly distinct from the competitive, reversible, active site inhibitors and is often distinguished from the NSAIDs. Aspirin and NSAIDs inhibit the COX enzymes and prostaglandin production; they do not inhibit the lipoxygenase path of AA metabolism and hence do not suppress LT formation.

Glucocorticoids suppress the induced expression of COX-2 and thus COX-2-mediated prostaglandin production. They also inhibit the action of phospholipase A₂, which releases AA from the cell membrane. These effects contribute to the antiinflammatory actions of glucocorticoids.

8.1.2. Anti-inflammatory agents from Natural source

Many medicinal plants have been traditionally used as source for anti-inflammatory agents. Some of them include Menthae piperitae aetheroleum, ß-glycyrrhetic acid from root of Glycyrrhiza glabra, Zingerone, which is formed from gingerol from rhizome of Zingiber officinale,cineole in the essential oil from the flowers of Achillea millefolium.

Matricaria recutita contain an essential oil rich in sesquiterpenes such as bisabolol and chamazulene which have excellent anti-inflammatory effects. Salicylic acid liberated from the Salicin-type glycosides of Populus tremuloides, by hydrolysis convert to the
aglycone-salicyl alcohol which is then oxidized in the liver to salicylic acid exhibit significant anti-inflammatory effect. Curcumin and its derivatives are the active anti-inflammatory constituents and its activity appears to be mediated through the inhibition of the enzymes trypsin and hyaluronidase.

8.2. MATERIALS AND METHODS

8.2.1. Materials

Combined ethyl acetate extract of stem bark of *Cissus quadrangularis* and fruit pulp of *Aegle marmelos* (c-EACA) and combined ethanol extract of stem bark of *Cissus quadrangularis* and fruit pulp of *Aegle marmelos* (c-ECA), Carrageenan, Indomethacin, Vehicle -1% tween 80, Oral needle.

8.2.2. Experimental Animals

the Wistar male albino rats weighing between 150-220 gm was procured from santhiram College of Pharmacy, Nandyal, Andhra Pradesh, India for the experiment. Which were maintained in a good aerated room with 12 hr light and dark cycle in polypropylene cages. Ethical committee clearance was done with Reference No. 1519/PO/a/11/CPCSEA.

8.3. EXPERIMENTAL DESIGN

Animals were separated into 6 groups. Each group consists of six rats.

**Group I** – received vehicle 1% Tween 80 (5 ml/kg, p.o) [Carragenean Control]

**Group II** – received Indomethacin (10 mg/kg, i.p) [Standard + Carragenean]

**Group III** – received combined ethyl acetate extract of stem bark of *Cissus quadrangularis* and fruit pulp of *Aegle marmelos* (c-EACA) 250 mg/kg, p.o

**Group IV** - received combined ethyl acetate extract of stem bark of *Cissus quadrangularis* and fruit pulp of *Aegle marmelos* (c-EACA) 500 mg/kg, p.o
Group V - received combined ethanol extract of stem bark of *Cissus quadrangularis* and fruit pulp of *Aegle marmelos* (c-ECA) 250 mg/kg, p.o

Group VI - received combined ethanol extract of stem bark of *Cissus quadrangularis* and fruit pulp of *Aegle marmelos* (c-ECA) 250 mg/kg, p.o

**Procedure**

The anti inflammatory activity of the c-EACA & c-ECA extracts were determined using carrageenan induced rat paw edema assay\(^5\). After 30 mins of single dose administration of the c-EACA & c-ECA extracts (250 & 500 mg/kg), 0.1ml of 1% carrageenan in 0.9% w/v of NaCl was injected into the sub plantar region of the left hind paw of each rat to induce edema for all groups. The paw volume was measured at intervals of 0, 15, 30, 60, 120 and 180 min after carrageenan injection by volume displacement method using Plethysmometer by immersing the paw in mercury cell. The percentage of inhibition of drug treated group was compared with control group. The percentage inhibition of paw edema was calculated by using the following formula;

\[
\text{Percentage of edema inhibition} = \left(\frac{V_c - V_t}{V_c}\right) \times 100
\]

\(V_c\) - Volume of paw edema in control group

\(V_t\) - Volume of paw edema in treated group

**Fig. 8.1: Development of Edema in paw of rat’s hind leg induced by 0.1ml of 1% carrageenan**
Statistical Analysis

The data were recorded as mean ± standard error mean (S.E.M). The significance of variations between the groups were calculated using one way and multiple way analysis of variance (ANOVA). The test followed by Dunnett’s test $P$ values less than 0.05 were noted as significance.

8.3. RESULTS AND DISCUSSION

Anti-inflammatory effect of c-EACA & c-ECA extracts were as significant at the level of $p<0.001$ when compared with the vehicle 1% tween 80 (control group) and indomethacin (Standard) (Table 8.1, Figure 8.2). The percentage of inhibition of paw edema after 3 hrs were recorded 67.19% in case of indomethacin, 55.02% and 61.90% in case of 250 mg/kg and 500 mg/kg of c-EACA and 56.61% and 64.02% in case of 250 mg/kg and 500 mg/kg of c-ECA respectively.

Anti-inflammatory activity was determined by using plethysmometer, one of the possible and frequently used animal model to screen anti-inflammatory drugs. The progress of carrageenan induced edema is bi-phasic, the first phase is release of histamine, serotonin and kinins and the second phase is the release of prostaglandins and bradykinins$^6$. 
Table 8.1: Effect of the c-EACA & c-ECA on carrageenan induced paw edema

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment &amp; Dose</th>
<th>Paw edema volume (ml)</th>
<th>Percentage of inhibition</th>
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<tr>
<td></td>
<td></td>
<td>0min</td>
<td>15min</td>
</tr>
<tr>
<td>I</td>
<td>1% Tween 80 (5ml/kg, p.o)</td>
<td>0.64±0.007 9</td>
<td>1.27±0.0145</td>
</tr>
<tr>
<td>II</td>
<td>Indomethacin, 10 mg/kg, i.p)</td>
<td>0.63±0.010 4</td>
<td>1.04±0.0129*</td>
</tr>
<tr>
<td>III</td>
<td>c-EACA 250mg/kg, p.o</td>
<td>0.62±0.010 7</td>
<td>1.18±0.0191*</td>
</tr>
<tr>
<td>IV</td>
<td>c-EACA 500mg/kg, p.o</td>
<td>0.64±0.011 7</td>
<td>1.12±0.0140*</td>
</tr>
<tr>
<td>V</td>
<td>c-ECA 250mg/kg, p.o</td>
<td>0.63±0.008 4</td>
<td>1.13±0.0135*</td>
</tr>
<tr>
<td>VI</td>
<td>c-ECA 500mg/kg, p.o</td>
<td>0.64±0.012 8</td>
<td>1.09±0.0149*</td>
</tr>
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Values are represented as mean ± SEM six observations. Comparison between Group I Vs Group II, III, IV, V & GroupVI

Statistical significant test for comparison was done by ANOVA, followed by Dunnett’s test. *p<0.05; ** p<0.01;
Fig. 8.2: Effect of the c-EACA & c-ECA on carrageenan induced paw edema
8.4. CONCLUSION

It was observed that c-EACA & c-ECA at the higher dose given i.e. 500 mg/kg possessed significant inhibition against carrageenan induced paw edema in rats. This response tendency of the c-EACA & c-ECA extracts in carrageenan-induced paw edema revealed good peripheral anti-inflammatory properties of the extract. Hence we suggest that c-EACA & c-ECA possess anti-inflammatory properties that may be due to presence of flavonoid and steroids potentiate the activity of extract.