INTRODUCTION

Inflammation is the immediate biochemical and cellular response of the body to cellular trauma in vascularized tissue. Within seconds of lethal or non-lethal cellular injury (i.e. mechanical trauma, oxygen or nutrient deprivation, microorganisms, extreme heat or cold, chemical agents, ionizing radiation) the cells and mediators of the innate or non-specific immune system initiate the process of inflammation. Regardless of the type of cellular injury, the process of inflammation occurs the same and thus exhibits the non-specific nature of the process. The clinical hallmarks of inflammation include rubor (redness), tumor swelling), calor (heat), dolor (pain), and functio laesa (loss of function). The goal of the cells and the proteins of the inflammatory system is to kill microorganisms, remove cellular and inflammatory derived debris and thus prepare the injured site for tissue regeneration or repair. One of the first results of inflammation is to ‘wall-off’ the area of injury from the remaining tissues. Fibrinogen clots block the tissue space and the lymphatic in the inflammed area so that after a while fluid rarely flows through the space. The process of walling-off delays the spread of bacteria or toxic products[272].

Acute inflammation is a self-limiting process that lasts about 8-10 days where as inflammation is considered chronic if it lasts longer than two weeks. Chronic inflammation can occur as a result of failed acute inflammatory processes or as a distinct process itself where the foreign substances are structured such that they are not easily phagoytosed or microorganisms that survive within macrophages (i.e., tuberculosis, syphilis) or due to persistent irritation by chemicals or physical irritants. Chronic inflammation is characterized by macrophage and lymphocyte infiltration. Granulomas are formed if neutrophils and macrophages are unable to kill the foreign substance during the acute phase of inflammation.
In response to inflammation, a sequence of events takes place. Mast cell degranulation involves in the release of vasoactive substances (i.e. histamine) that stimulates an immediate vasoconstriction followed by a vasodilation that increases blood flow to the area which aids the formation of exudates, and release of chemotactic factors that provide a chemical gradient for the guidance of neutrophils and eosinophils to the inflamed site. Endothelial cell retraction allows for increased permeability. WBCs squeeze through these capillary spaces by the process of diapedesis. Neutrophils are the first of the phagocytic leukocytes to arrive at the inflamed area. The neutrophils phagocytose (ingest) bacteria, dead cells, and cell debris. These phagocytic cells are short lived and are removed as pus through the epithelium or removed by the lymphatics. Macrophages (derived from monocytes) are the next phagocytes to arrive in the inflamed area where they function as phagocytes for a much longer time than neutrophils. Additionally macrophages perform other important functions necessary for the initiation and function of the immune system. These functions include processing and presenting antigen to the lymphocytes, release of chemicals (nitrous oxide, prostaglandins) and secretion of cytokines. In addition, emigration of eosinophils (for protection against parasites) and basophils (circulatory functional counterpart to mast cells) occurs. Platelets are also found in the inflamed area. They release the vasoactive substance serotonin and play a key role in the clotting mechanism[273]-[279].

**Mediators of inflammation**[280]-[288]:

Mast cells are found in the tissues. Their cytoplasm is loaded with granules containing mediators of inflammation. Their surface is coated with a variety of receptors which, when engaged by the appropriate ligand, trigger exocytosis of the granules. Mast cells appear to be key players in the initiation of inflammation. Activated mast cells release literally dozens of potent inflammatory mediators like chemokines, histamine, bradykinin, inflamasomes, interleukins, prostaglandins, leukotrienes and reactive oxygen species (ROS). These mediators are active in recruiting all the types of white blood cell to the site and activating many of these recruited cells to produce their own mediators of inflammation.
**Chemokines:**

Chemotactic cytokines are secreted proteins that attract other leukocytes into the area.

**Reactive Oxygen Species (ROS):**

These are produced by activated phagocytes, macrophages and neutrophils. They are toxic for microorganisms but can also lead to tissue injury.

**Histamine:**

The granules of mast cells are loaded with histamine and their exocytosis releases this potent mediator. Histamine increases the blood flow to the area and the leakage of fluid and proteins from the blood into the tissue space. Thus the quick release of histamine produces the redness and swelling associated with inflammation.

**Interleukin-1 (IL-1):**

Macrophages, monocytes, and activated platelets are sources of this cytokine. IL-1 has both

- paracrine effects on cells in the vicinity, e.g.,
  - causing them to produce tissue factor and thus triggering the blood clotting cascade
  - stimulating the synthesis and secretion of a variety of other interleukins
  - helping to activate T cells and thus initiate an adaptive immune response
- endocrine (hormonal) effects as it is carried in the blood throughout the body.
  - decreasing blood pressure
  - inducing fever.
IL-1 causes fever by stimulating the release of prostaglandins, which act on the temperature control center of the hypothalamus.

**Inflammasomes**

IL-1 is synthesized from a larger precursor that is cleaved by caspase-1. Caspase-1 is part of two (or more) multi-protein complexes in the cytosol of macrophages and neutrophils that are called inflammasomes. Inflammasomes are activated by several different products produced by invading bacteria. These provide a link between the innate immune system and inflammation.

**Bradykinin**

Bradykinin is a nonapeptide (9 amino acids). It is synthesized by proteolytic cleavage of an inactive precursor (a kininogen) produced by the liver and circulates at all times in the blood. Bradykinin relaxes the smooth muscle walls of the arterioles, lowers blood pressure, increases blood flow to the tissue and makes the capillaries to leak, allowing blood components to enter the tissue space. These effects (like those of histamin) produce the redness, warmth, and swelling of inflammation. It also stimulates phospholipase to increase the production of prostaglandins and the release of nitric oxide.

**Prostaglandins and Leukotrienes:**

These potent mediators of inflammation are derivatives of arachidonic acid (AA), a 20-carbon unsaturated fatty acid produced from membrane phospholipids. The principal pathways of arachidonic acid metabolism are the cyclooxygenase (COX) pathway, which produces prostaglandin H2 (PGH2) and the 5-lipoxygenase pathway, which produces a collection of leukotrienes (LT). PGH2 serves as the substrate for two enzymatic pathways: one leading to the production of several prostaglandins (PG); the other leading to the production of thromboxanes (Tx).
Anti-inflammatory agent is a drug that inhibits any facet of inflammation of an experimentally induced nature or as a part of clinical syndrome\textsuperscript{[289]}. Aspirin (LVII) and sodium salicylate (LVIII) have been widely used as remedial drugs for inflammation. Intensive research on steroidal drugs was based on the remarkable anti-inflammatory activity shown by synthetic samples of corticosteroids\textsuperscript{[290]}. But the hormonal and metabolic side effects of those steroidal drugs could not be reduced\textsuperscript{[291]}. This led to the development of non-steroidal anti-inflammatory drugs.

The mechanism of action of non-steroidal anti-inflammatory drugs lies in their ability either to inhibit the synthesis or to block the activity of prostaglandins which mediate the inflammatory response\textsuperscript{[292]}. As a result of an extensive study on the anti-
inflammatory activity of flavonoids, it has been established that many members of this category of compounds do exhibit significant activity\(^{293}\).

The ethereal fraction of methiseed, *Trigonella foenum-graceum* has been shown to be effective in chronic inflammation model and the activity was comparable to that of sodium salicylate\(^{294}\). These workers have also demonstrated that the aq. extract of *Ipomea turpethum* was effective in rats\(^{295}\). Similarly, the anti-inflammatory activity has been reported for the oils of *Ricinus communis* and *Sesamum indicum*\(^{296}\). The crude extract of *Ephedra intermediae* has been shown to possess anti-inflammatory activity\(^{297}\). The aq. extracts of *Tinospora cordifolia* has been found to inhibit carrageenan induced oedema in rats while it has been effective in granuloma pouch method\(^{298}\).

The leaves of *Streblus asper*\(^{299}\) and *Argemone Mexicana*\(^{300}\) have been observed to be active against carrageenan induced rat paw oedema in rats and their activities are comparable to that of phenylbutazone (LIX). The methanolic extract of *Acanthus ilicifolius* has been reported\(^{301}\) to show anti-inflammatory activity in carrageenan oedema.

The plant derived flavonoid glycosides have been tested for their anti-inflammatory activity against carrageenan - induced rat paw oedema and the results are presented hereunder.

**MATERIALS AND METHODS**

For the investigation of anti-inflammatory activity of the flavonoid glycosides, rat paw oedema method is most commonly used. It is an acute experiment in which a noxius agent is injected into the sub-plantar region of the rat’s paw. Within a few seconds after the injection, an oedematous swelling is observed at the foot. This swelling remains for a few hours\(^{302}\). The anti-inflammatory activity of the isolated glycosides was evaluated using carrageenan induced rat-paw oedema\(^{303}\). Carrageenan oedema is suitable for investigating anti-inflammatory drugs since it in
little affected by drugs with other pharmacological functions and oedema produced is less affected by non-specific factors such as vasodilation, ganglion blockage or diuresis. Of the various techniques available plethsmography method probably gives the most reliable results and allows a rapid and reproducible quantitative assessment of swelling[304],[305].

**Reagents and Chemicals:**

All the chemicals and reagents used were purchased from SISCOM chemicals Ltd., Tiruchirappalli, India.

**Animals:**

Male albino wistar rats (15 – 20 weeks old) were purchased from Sri Venkateswara Agencies, Bangalore, India. The rats were acclimatized for one week to a balanced diet (Crude Protein – 22.3 %, Crude Oil – 4.01 %, Crude fibre – 4.02 %, Ash – 8.02 % and Sand Silica – 1.02 %), with food and water and were maintained in a 12/12 h. light/dark cycle.

**EXPERIMENTAL**

Young, male adult rats (Tamil University – animal house – registration number being: 791/03/B/CPCSEA), weighing about 150-200 g were fed with normal food and water before and during the experiments seven groups of rats (five in each group) were used. All the drugs were given intraperitoneally. The first group was fed with normal saline and served as control, the second group was given phenylbutazone in doses of 100 mg/kg BW while the last 5 groups were administered with an aqueous solution of the isolated flavonoid glycosides in doses of 25 mg/kg BW.

A solution of Carrageenan (Viscarin red, 7028), 1% (w/v) in 0.9% (w/v) sterile saline solution was prepared and 0.1 ml of it was injected 30 min later into the sub-plantar tissue of the left hind paw. The volume of the hind
upto the tibiotarsal articular region noting the displacement of lower mercury column in plethysmometer\textsuperscript{306}. The results were expressed as increase in foot volume in ml over the initial volume. The volume was measured in the 1\textsuperscript{st} h, 2.5 h and 5.5 h intervals.

\[
\text{Percentage of anti-inflammatory activity} = \frac{\text{The volume of oedema of drug treated group}}{\text{The volume of oedema of control group}} \times 100
\]

The effect of the isolated flavonoids on carrageenan induced rat-paw oedema is accounted in table II.

**Statistical analysis\textsuperscript{308}:**

Bio-chemical values are expressed as mean ± standard error of mean (SEM) of untreated controls and drug treated groups separately.

Where,

\[
\text{SEM} = \frac{\text{SD}}{\sqrt{n}}
\]

\[
n = \text{number of samples}
\]

Statistical analysis of the values obtained were done using students ‘t’ test to arrive at the significance of the difference of two means of treated groups and control group. The deviation of ‘t’ by means of which the probability ‘p’ is obtained is calculated by the formula,

\[
t = \frac{\text{x}_1 - \text{x}_2}{\text{SD}}
\]

Where,

\[
\text{x}_1 - \text{x}_2 \text{ is the difference between the means and SD, the standard deviation of the difference between means. }\text{S}^2\text{D for small samples, where degree of freedom (d.f) } n_1 + n_2 - 2 < 60 \text{ is calculated as follows.}
\]

\[
\text{SD} = \text{____________}
\]
Where,

\[ S^2_{x_1x_2} \text{ or Common Variance} = \ldots \]

\[ S \], \text{ using respective sum of squares of deviations, } p > 0.05 \text{ is considered as statistically not significant and } p < 0.001 \text{ as highly significant difference between the biochemical values of untreated control group and drug treated groups.} \]
### TABLE - II

**EFFECT OF ISOLATED FLAVONOIDs ON CARRAGEENAN INDUCED RAT PAW ODEMA**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Groups*</th>
<th>Dose (mg/kg BW)</th>
<th>Odema volume (ml ±S.E.) + 1 h.</th>
<th>% anti-inflammatory activity</th>
<th>Odema volume (ml ±S.E.) + 2.5 h.</th>
<th>% anti-inflammatory activity</th>
<th>Odema volume (ml ±S.E.) + 5.5 h.</th>
<th>% anti-inflammatory activity</th>
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<td>1</td>
<td>Control</td>
<td>--</td>
<td>0.42±0.04</td>
<td>--</td>
<td>0.45±0.03</td>
<td>--</td>
<td>0.50±0.02</td>
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<td>2</td>
<td>G1</td>
<td>50</td>
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<td>21.4</td>
<td>0.23±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>48.8</td>
<td>0.25±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>50.0</td>
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<tr>
<td></td>
<td></td>
<td>100</td>
<td>0.26±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>38.1</td>
<td>0.21±0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>53.3</td>
<td>0.20±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>60.0</td>
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<tr>
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<td></td>
<td>200</td>
<td>0.28±0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>33.3</td>
<td>0.25±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>44.4</td>
<td>0.29±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>55.5</td>
<td>0.21±0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>58.0</td>
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<td>23.8</td>
<td>0.26±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>42.0</td>
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<td>48.0</td>
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<tr>
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<td>40.0</td>
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<td>0.23±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>54.0</td>
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<td>33.3</td>
<td>0.25±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>44.4</td>
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<td>0.20±0.02&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>Phenyl Butazole</td>
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<td>0.19±0.03</td>
<td>57.8</td>
<td>0.20±0.04</td>
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*Groups:*
Ethyl acetate concentrates of,
The number in the parentheses indicates the percentage anti-inflammatory activity.
Values are mean ± SD of 5 animals in each group.
All groups were compared with Control, *p < 0.05;  p < 0.01*
NS – Statistically not significant
Fig. II-1 - EFFECT OF ISOLATED FLAVONOIDS ON CARRAGEENEN INDUCED RAT PAW ODEMA AT 1 HOUR
Fig. II-2 - EFFECT OF ISOLATED FLAVONOIDs ON CARRAGEENEN INDUCED RAT PAW ODEMA AT 2.5 HOURS
Fig. II-3 - EFFECT OF ISOLATED FLAVONOIDS ON CARRAGEENEN INDUCED RAT PAW OEDEMA AT 5.5 HOURS
RESULTS AND DISCUSSION

Carrageenan induced inflammation is an acute inflammation\textsuperscript{[308]}. The oedema which develops after carrageenan injection is a biphasic event\textsuperscript{[309]}. The initial phase is attributed to the release of histamine and serotonin. The oedema maintained between the first and second phase is due to the kinin-like substances. The second phase is said to be promoted by prostaglandin-like substances. The recognition of different mediators in different phases of oedema has important implications. It has been reported that the second phase of oedema is sensitive to drugs like hydrocortisone, phenylbutazone and indomethacin\textsuperscript{[310]}.

From Table II, it can be observed that the aq. solutions of the flavonoid glycosides isolated in chapter I, at a dose of 100 mg/kg BW are able to inhibit carrageenan induced oedema at different intervals of time better than other doses. Phenylbutazone, a standard NSAID at a dose of 100 mg/kg BW is able to control carrageenan induced inflammation at all the intervals of time. The carrageenan assay is suited for the comparative bioassay of anti-inflammatory agents. All these drugs are nearly able to control the second phase at 5.5 h mediated by prostaglandins. So it is likely that the anti-inflammatory effect of these drugs may be due to the inhibition of prostaglandins as in the case of standard anti-inflammatory drug phenylbutazone.