CHAPTER IV
ANTI-INFLAMMATORY ACTIVITY OF FLAVONOIDS

INTRODUCTION

Inflammatory reaction is a basic defensive response to a variety of forms of stimuli which may be biological, chemical or physical\textsuperscript{410}. This reaction can be induced by a number of chemical mediators including kinins and histamine; but recent evidence suggests that prostaglandins may be of particular importance in many types of inflammation. They have been detected in exudates from experimentally induced inflammation and were found when perfusion studies were carried out in skin lesions of allergic volunteers with contact dermatitis\textsuperscript{411}.

The term inflammation originates from Lat: 'inflammare' meaning 'to burn'. Inflammation is a homeostatic phenomenon which is regarded as a wholesome response to an irritant. The irritant provokes one after another the mechanisms that combat damage and inflammation subsides when it is no longer
needed. The clinical signs that inflammation evoke are heat, redness, swelling and loss of function.

Inflammation may be broadly classified under two categories viz. acute and chronic inflammations. The acute inflammation is the response of tissues to severe but transient stimuli. It is a predominantly vascular process characterised by increased tendency for fluid and plasma proteins to pass through the vessel wall into extra vascular space (vascular permeability), leucocytic infiltration and pain.

The chronic or granulomatus inflammation occurs when a stimulus is persistent. It is a process which gradually replaces the acute condition when the irritant happens to be a mild substance and is characterised by cellular infiltration which is more heterogeneous than that of acute inflammation. The appearance of a number of monocytes, macrophages, histocytes and fibroplasts at the site of injury is a characteristic feature of the inflammation. Phagocytic cells migrate into the area and cellular lysosomal membranes may be ruptured releasing lytic enzymes.
Lysosomal enzymes play an important role in the development of acute and chronic inflammation. Increased enzyme activity has been reported in certain types of experimental inflammation.

'Anti-inflammatory agent' is a drug that inhibits any facet of inflammation of an experimentally induced nature or as a part of a clinical syndrome.

Screening procedures to evaluate the anti-inflammatory drugs are classified into four categories: (i) an interference with the manifestation of one of the cardinal signs of inflammation; (ii) the modification of one of the events occurring during the inflammatory process; (iii) biological or chemical properties of known anti-inflammatory drugs; (iv) the modifications of those syndromes in laboratory animal models which are considered to represent models for various rheumatic diseased states.

Aspirin and sodium salicylate have been widely used as remedial drugs for
LXXIV
Aspirin

LXXV
Sodium salicylate

LXXVI
Phenyl butazone

LXXVII
Indomethacin
inflammation. Intensive research on steroidal drugs was based on the remarkable anti-inflammatory activity shown by synthetic samples of corticosteroids$^{417}$. But the hormonal and metabolic side effects of those steroidal drugs could not be reduced$^{418}$. 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$^{419}$. The inhibitory effects of non-steroidal anti-inflammatory drugs on lysosomal enzymes have been cited as responsible for their mode of action$^{420}$. Anti-inflammatory agents like phenylbutazone(LXXVI) and indomethacin(LXXVII) have been shown to exert their beneficial effects by inhibiting the activities of either released lysosomal enzymes or by stabilizing the lysosomal membrane$^{421}$. Many other anti-inflammatory drugs have been reported to stabilize the lysosomal membrane as also to inhibit the lysosomal enzyme$^{422}$. 
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\textsuperscript{423}. It has been reported that rutin, tri(hydroxyethyl)-rutin and magnesium flavonic chelates exert \textit{in vitro} stabilizing effect on the lysosomal membranes\textsuperscript{424}.

It has been reported that the structure of red blood corpuscles (RBC) is similar to lysosomal membrane components. Since lysosomal membrane resembles human RBC membranes, its stabilization effects have been studied using HRBC\textsuperscript{425}. When the RBC is subjected to hypotonic stress, the release of haemoglobin from RBC is prevented by anti-inflammatory agents because of membrane stabilization. So, the stabilization of HRBC membrane by drugs against hypotonicity-induced haemolysis serves as a useful \textit{in vitro} method for assessing the anti-inflammatory activity of various compounds\textsuperscript{426}.

Flavonoids like quercetin, rutin, hyperoside, naringenin and naringin have been reported to exert \textit{in vitro} stabilizing action on the HRBC membrane.
against hypotonicity-induced haemolysis. The roots of *Withania somnifera* have been established to stabilize the RBC lysosomal membrane systems.

Luteolin, its 7-0-glucoside and genistein isolated from the *Genista* spp., the alcoholic extract of *Cleodendrom inerme* containing the glycosides of apigenin and scutellarein, the flavonoids of *Azadirachta indica* and *Anacardium occidentale* and the alcoholic extract of *Physalis minima* have been reported to exhibit anti-inflammatory activity.

Anti-inflammatory activity shown by *Indigofera aspalathoides* has been correlated to the presence of polyphenolics. Bavachinin (LXXVIII) obtained from *Psoralea corylifolia* has also been shown to possess anti-inflammatory activity. Vitexin isolated from *Ochnocarpus longifolius* and *Arnebia hissidissima* possessed a moderate anti-inflammatory activity in addition to potent hypotensive activity. Apigenin 5,7-dimethylether (LXXIX) obtained from *Rhus*
$\text{LXXVIII}$

$R = \text{Me, Bauachinin}$

$\text{LXXIX}$

Apigenin 5,7-di-O-methylether

$\text{LXXX}$

Liquiritigenin
undulata\textsuperscript{436} and bergenin isolated from the flowers of \textit{Peltophorum pterocarpum}\textsuperscript{437} have been shown to possess significant anti-inflammatory effect.

\textit{In vitro} haemolytic studies on the flowers of \textit{Sesbania grandiflora} have been reported\textsuperscript{438}. Hypolaetin 8-0-glucoside(LXVIIIb) obtained from \textit{Sideratis mugronesis} has been recorded to exhibit anti-inflammatory activity comparable to that of phenylbutazone in acute and chronic phases of inflammation\textsuperscript{439}. Three flavonoids viz. baicalein, baicalin(LXVIIIc) wogonin(LXVIIIId) isolated from \textit{Scutellaria baicalensis}\textsuperscript{440} and chrysoeriol 7-0-\(\beta\)-D-glucopyranosyl (2 \(\rightarrow\) 1) D-apiofuranoside(XXXVIIIj) isolated from \textit{Dalbergia volubilis}\textsuperscript{441} have been reported to exhibit anti-inflammatory activity. The effect of eleven flavonoids and four biflavonoids on the release of histamine, which is a mediator of inflammation has been investigated\textsuperscript{442}. Anti-inflammatory and anti-histaminic activities of \textit{Datura stramonium} containing kaempferol and quercetin has been reported\textsuperscript{443}. Recently chrysin(LVIf), liquiritigenin(LXXX) and naringenin have also been
isolated from the plant extract. There is a recent report on the anti-inflammatory activity of the leaf extract of Cassia alata.

The pharmacotherapy of inflammation is characterised by an apparent abundance of synthetic drugs which often have dangerous side effects. The situation calls for intensive efforts to search for more specific and less toxic anti-inflammatory compounds and has necessitated large scale screening of many plants. Scientists at the Central Drug Research Institute, Lucknow have identified as many as 41 medicinal plants possessing significant anti-inflammatory activity.

During the present work the in vitro anti-inflammatory activity of the flavonoid glycosides isolated from various Indian plants listed in Chapter I has been evaluated by studying the HRBC membrane stabilization by the drugs against hypotonicity-induced haemolysis.
EXPERIMENTAL

Fresh blood was collected from healthy male adult human volunteers and mixed with equal volume of sterilised alsever solution containing 2% dextrose, 0.8% sodium citrate, 0.05% citric acid and 0.42% NaCl and used within 5 h. Hyposaline (0.36%, 2 ml), phosphate buffer (0.15 M, pH = 7.4, 1 ml) and HRBC (1%, 0.5 ml) were taken in four tubes. Solutions of different concentrations of the drug were added in three of the above tubes. The fourth tube served as control in which instead of the drug, isosaline (0.85%, 1 ml) was added. The contents in all the four tubes were incubated at 37°C for 30 min and then centrifuged. The intensity of colour of the supernatant which was due to haemoglobin was measured at 560 nm in a 430 B Double beam spectrophotometer. The control tube that contained no drug was taken as representing 100% HRBC lysis. The percentage of prevention of drug-treated hyposaline-induced HRBC lysis was calculated from the difference in absorbance readings of the control and drug-treated tubes using the following relation:
The results are depicted in Table IV-1.

RESULTS AND DISCUSSION

The HRBC membrane stabilization effects of the isolated bioflavonoids can be inferred from the data presented in Table IV-1, which show a dose-dependent activity against haemolysis. Many of the flavonoids tested have maximal inhibition of hypotonicity induced HRBC lysis at a concentration of 50 or 75 µg/ml.

Hypotonic solution induces the HRBC lysis. The principle behind this is that the hypotonic solution will enter into RBC as a result of difference in osmotic pressure, by endosmosis, RBCs swell in size to a certain extent and will finally burst allowing the haemoglobin to leak out. The haemoglobin content in the supernatant solution is then estimated.
<table>
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<th>Drug*</th>
<th>Dose</th>
<th>µg/ml</th>
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<tbody>
<tr>
<td></td>
<td>10</td>
<td>25</td>
</tr>
<tr>
<td>G_2</td>
<td>32.6</td>
<td>42.8</td>
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<tr>
<td>G_3</td>
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<td>50.1</td>
</tr>
<tr>
<td>G_8</td>
<td>12.5</td>
<td>52.0</td>
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**TABLE IV-1**

Effect of Flavonoids against Hypotonicity Induced Haemolysis

(\% prevention)
<table>
<thead>
<tr>
<th>Drug*</th>
<th>Dose g/ml</th>
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<tr>
<td></td>
<td>10</td>
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<tr>
<td>G₉</td>
<td>18.1</td>
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<tr>
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<td>28.5</td>
</tr>
<tr>
<td>G₁₂</td>
<td>50.5</td>
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</tbody>
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*Key to drugs:

G₂ - Rutin
G₃ - Kaempferol 3-0-[(6"-0-acetyl)-gluco-7-0-rhamnoside
G₄ - Luteolin 7-0-rutinoside
G₅ - Patuletin 3-0-glucuronide
G₆ - Isorhamnetin 3-0-sophoroside
G₇ - 3',4',6,8-Tetrahydroxyflavonol-5'-methylether-7-0-neohesperidoside
G₈ - Apigenin 7-0-glucorhamnolabinoside
G₉ - Quercetin 5-0-rhamno-3'-0-arabinoside
G₁₀ - 3',4'-Dihydroxyflavone-7-0-glucuronide
G₁₁ - Hispidulin 7-0-neohesperidoside
G₁₂ - Myricetin 5'-methylether-3-0-neohesperidoside
Non-steroidal anti-inflammatory drugs (NSAIDs) like flavonoids decrease the release of marker enzymes and hence stabilize the hepatic lysosomes\textsuperscript{450}. RBC membrane system is similar to lysosomal membrane system\textsuperscript{451}. It is suggested that NSAIDs interact with the biological membrane \textit{in vitro}, the proteins being the main binding sites for these drugs within the biological membrane\textsuperscript{452}. Hence the HRBC membrane stabilizing effects may be due to the binding of the active principle of the flavonoid glycosides with the proteins or phospholipids of the membrane\textsuperscript{453}. It may be inferred that these glycosides may exert their anti-inflammatory activity by stabilizing the lysosomal membrane system.

From these observations, it can be concluded that the flavonoid glycosides isolated from the fresh flowers of different plants mentioned in Chapter I have considerable anti-inflammatory activity.