Chapter VI

EFFECTS OF INDIGENOUS ANTI-INFLAMMATORY DRUGS ON LYSOSOMAL STABILITY

VI.1 Introduction

Lysosomes are heterogeneous group of cytoplasmic membrane-bound organocelles that mediate the digestive and lytic processes of the cell. The lysosomes have been implicated in a variety of biological functions such as regulation of secretory processes, cellular defence mechanisms, cell death, protein and organelle turnover, accumulation and sequestration of xenobiotics and mediation of target tissue specific hormone action. Lysosomes contain several enzymes—mostly hydrolytic, a number of cationic proteins and probably one anionic protein which may be involved in the inflammatory response. Agents (toxins) which disrupt cell biomembranes release cellular constituents including lysosomal enzymes into surrounding tissue or cytoplasm which may further provoke inflammation and tissue injury. Lysosomal enzymes can completely degrade the components of connective tissue such as collagen, protein mucopolysaccharide complexes,
glycoproteins and elastin. Drugs by stabilizing the membrane, can prevent the rupture of the lysosomes and inhibit the release of lysosomal enzymes. Most of the steroidal and non-steroidal anti-inflammatory drugs stabilize lysosomal membranes in vitro and in vivo. The lysosomal enzymes are implicated in the pathogenesis of articular tissue degradation in several rheumatic diseases. The injury caused by enzymes released from lysosomes and the modification of normal tissue constituents to antigenic forms by the mediation of these enzymes are factors affecting inflammatory disorders.

Pilero and Colombo observed increased acid phosphatase activity in serum as well as homogenate of inflamed paw during adjuvant-induced polyarthritis. Similarly increased lysosomal enzyme activity has been reported in certain types of rat paw edema produced by a phlogistic agent. Non-steroidal anti-inflammatory drugs are known to inhibit the release of lysosomal enzymes from lysosomes and to stabilize lysosomal membrane.

Arrigoni-Martelli et al. reported that nystatin induces inflammatory reaction by labilizing the lysosomal membranes and this reaction is inhibited by both steroidal and non-steroidal anti-phlogistic drugs which have been shown to have stabilizing effects on lysosomal membranes both in vitro and in vivo. Jain et al. showed that Brahmi Rasayan [an ayurvedic medicinal mixture, whose major constituent is Brahmi (Bacopa monniera) leaves] has caused significant inhibition of nystatin-induced edema. Their study provides an indirect evidence that like other anti-inflammatory drugs it may induce this effect by stabilizing the lysosomal membranes.

Cortisol and its analogues are reported to stabilize the membranes of lysosomes both in vivo and in vitro in experimental animals.
Alterations in lysosomal function and stability have been implicated in several disorders. The lysosomal system is observed to respond to physiological and pathological changes and a variety of physical and chemical agents.

VI.2 Materials and Methods

The rabbits were divided into six groups as described in Chapter V. They were female control (group I), roots of *Sida retusa* Linn. treated female (group II), rhizome of *Curcuma longa* Linn. treated female (group III), male control (group IV), the whole plant of *Hydrocotyle asiatica* Linn. treated male (group V) and leaves of *Alstonia scholaris* Linn. treated male (group VI).

The animals were maintained on the same diet as described in Chapter V. Water was fed ad lib. The animals of the treated groups were administered daily the respective herb, as described in Chapter V for 5½ months.

At the end of the experimental period, the animals were sacrificed and the tissues were removed to ice cold containers immediately. The liver was homogenized in 0.25M sucrose solution at 0°C to prepare a 20% homogenate.

The activity of β-glucuronidase (E.C. 3.2.1.31), a lysosomal marker enzyme, was assayed following the procedure of Kawai and Anno using p-nitrophenyl-β-D-glucuronide as substrate.

p-nitrophenyl-β-D-glucuronide in 0.1M acetate buffer (pH = 4.5) was incubated with the enzyme at 37°C for 30 minutes. The reaction was arrested by adding 0.2N sodium carbonate solution. The absorbance was measured at 400 nm. The enzyme activity is expressed as milligrams of p-nitrophenol
liberated per hour per g. protein. The liver cell homogenate was subjected to
differential centrifugation in a refrigerated centrifuge. The homogenate was
centrifuged at 600 x g for 10 minutes and the sediment of nuclei, unbroken
cells and plasma membrane (nuclear fraction) separated. The supernatant
was then centrifuged at 15,000 x g for 30 minutes and the sediment
(lysosome-rich fraction) separated and the supernatant collected. The
15,000 x g supernatant (soluble fraction) was diluted with an equal volume of
0.2M acetate buffer (pH = 4.5). The nuclear and lysosome-rich fraction were
resuspended in 0.1M acetate buffer (pH = 4.5) containing 0.1% Brij-35. The
activity of β-glucuronidase, a typical lysosomal marker enzyme, was
determined in the nuclear, lysosome-rich and the soluble fractions. The total
β-glucuronidase activity of the tissue was determined by homogenizing in
0.1M acetate buffer (pH = 4.5) containing 0.1% Brij-35. The concentration of
Brij-35 in the final medium was less than 0.025% in every assay. Protein was
estimated in the tissue homogenates by the method of Lowry et al. Statistical
analysis was carried out using Student's 't'-test.

The ratio of soluble activity to lysosomal activity was determined in
each group. It is used as an index of lysosomal lability.

VI.3 Results and Discussion

The results are given in Tables VI.1a and VI.1b. It was observed that
there was no significant change in the total specific activity of β-glucuronidase
in the liver, on treatment with the above mentioned indigenous drugs.

The activity of β-glucuronidase was not significantly affected in the
nuclear fraction of the treated groups when compared to the control groups.
The activity of β-glucuronidase in the hepatic lysosomal fraction was also not changed significantly by the oral administration of the selected indigenous drugs.

The activity in hepatic soluble fraction (15,000 x g supernatant) was least in the group receiving the whole plant of *Hydrocotyle asiatica* Linn. It was significantly less than that of control in the group treated with aqueous extract of roots of *Sida retusa* Linn. The change was insignificant in the case of groups treated with rhizome of *Curcuma longa* Linn. or leaves of *Alstonia scholaris* Linn., compared to the respective control groups.

The ratio was considerably lower in the groups receiving the whole plant of *Hydrocotyle asiatica* Linn., roots of *Sida retusa* Linn. and rhizome of *Curcuma longa* Linn., when compared to the respective control group. The ratio was not appreciably affected in the animals treated with leaves of *Alstonia scholaris* Linn.
Table VI.1a. **Total and subcellular distribution of beta-glucuronidase in liver**

(Values are mean ± S.E.M. from 10 animals in each group)

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Total activity in liver</th>
<th>Activity in hepatic nuclear fraction</th>
<th>Activity in hepatic lysosomal fraction</th>
<th>Activity in hepatic soluble fraction</th>
<th>Ratio of soluble to lysosomal activity in liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Female control</td>
<td>70.17 ± 1.61</td>
<td>4.49 ± 0.10</td>
<td>42.70 ± 0.91</td>
<td>17.28 ± 0.38</td>
<td>0.405</td>
</tr>
<tr>
<td>II</td>
<td>Roots of <em>Sida retusa</em> Linn. (Kurumthotty)</td>
<td>64.88 ± 1.99</td>
<td>4.15 ± 0.14</td>
<td>42.67 ± 1.49</td>
<td>15.26 ± 0.47*</td>
<td>0.358</td>
</tr>
<tr>
<td>III</td>
<td>Rhizome of <em>Curcuma longa</em> Linn. (Manjal)</td>
<td>68.84 ± 2.20</td>
<td>4.37 ± 0.18</td>
<td>41.67 ± 1.33</td>
<td>16.37 ± 0.56</td>
<td>0.393</td>
</tr>
<tr>
<td>IV</td>
<td>Male control</td>
<td>70.29 ± 2.20</td>
<td>4.35 ± 0.14</td>
<td>43.21 ± 1.38</td>
<td>17.30 ± 0.54</td>
<td>0.400</td>
</tr>
<tr>
<td>V</td>
<td>The whole plant of <em>Hydrocotyle asiatica</em> Linn. (Kudakan)</td>
<td>66.30 ± 1.37</td>
<td>4.27 ± 0.09</td>
<td>46.22 ± 1.29</td>
<td>11.77 ± 0.25*</td>
<td>0.255</td>
</tr>
<tr>
<td>VI</td>
<td>Leaves of <em>Alstonia scholaris</em> Linn. (Pala)</td>
<td>69.92 ± 3.13</td>
<td>4.38 ± 0.11</td>
<td>43.14 ± 0.99</td>
<td>17.51 ± 0.46</td>
<td>0.406</td>
</tr>
</tbody>
</table>

*a = p<0.01  
b = 0.01<p<0.05  
no symbol = not significant
### Table VI.1b. **t-values**

<table>
<thead>
<tr>
<th>Groups compared</th>
<th>t-value between groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total activity in liver</td>
</tr>
<tr>
<td>I and II</td>
<td>1.96</td>
</tr>
<tr>
<td>I and III</td>
<td>0.46</td>
</tr>
<tr>
<td>IV and V</td>
<td>1.46</td>
</tr>
<tr>
<td>IV and VI</td>
<td>0.09</td>
</tr>
</tbody>
</table>
The ratio of soluble (released or free) to lysosomal (bound) \( \beta \)-glucuronidase activity is an index of lysosomal lability.\(^{459,460}\) Of the four herbal extracts investigated, all except leaves of *Alstonia scholaris* Linn. and rhizome of *Curcuma longa* Linn. exerted remarkable lysosome-stabilizing action. The order of decreasing lysosomal-stabilizing action is:

the whole plant of *Hydrocotyle asiatica* Linn. (0.255) > roots of *Sida retusa* Linn. (0.358) > rhizome of *Curcuma longa* Linn. (0.393).

The possible metabolic modifications of the pharmacological principles and their intracellular level are factors affecting their effect on lysosomal stability in vivo. The present study indicates that the lysosome-stabilizing action of the whole plant of *Hydrocotyle asiatica* Linn. and roots of *Sida retusa* Linn. fed orally may be involved in their reported anti-inflammatory action. The pharmacological potential of these indigenous herbal principles appear to be remarkable and warrants detailed investigations.