Section B

BIOCHEMICAL STUDIES

(1) EFFECT ON PROTEIN CONTENT:
(a) Effect on serum and liver protein content in cotton pellet implanted rats.

**Serum protein content**: The data have been summarised in Table -20. Significant increase in serum protein content was observed in cotton pellet implanted rats in comparison to normal control. In both ME of VAL and ME of VNDL treated groups, serum protein significantly decreased in comparison to cotton pellet implanted (CP) control rats at both the dose levels studied. In reference standard groups, no significant change was observed in BET administered groups. However, significant decrease was noted in PBZ treated group.

**Liver protein content**: Cotton pellet implantation did not affect liver protein content significantly in comparison to normal control rats. Significant increase in liver protein content was observed in ME of VAL, lower dose ME of VNDL and BET administered groups. No significant change could be observed in higher dose, ME of VNDL and PBZ treated groups.

**TABLE - 20**

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Serum</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mg/dl</td>
<td>mg/g wet tissue</td>
</tr>
<tr>
<td>1.</td>
<td>Normal control</td>
<td>5.95±0.37</td>
<td>3.09±0.19</td>
</tr>
<tr>
<td>2.</td>
<td>C.P. Control</td>
<td>7.75±0.70*</td>
<td>2.41±0.36</td>
</tr>
<tr>
<td>3.</td>
<td><em>Vitex altissima</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>100</td>
<td>5.47±0.20*</td>
<td>6.22±0.16***</td>
</tr>
<tr>
<td>5.</td>
<td>200</td>
<td>5.63±0.10*</td>
<td>8.58±1.02***</td>
</tr>
<tr>
<td>6.</td>
<td><em>Vitex negundo</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>200</td>
<td>5.90±0.09*</td>
<td>3.62±0.06**</td>
</tr>
<tr>
<td>8.</td>
<td>400</td>
<td>5.06±0.09*</td>
<td>3.20±0.22</td>
</tr>
<tr>
<td>9.</td>
<td>Phenylbutazone</td>
<td>4.73±0.36**</td>
<td>3.42±0.43</td>
</tr>
<tr>
<td>10.</td>
<td>100</td>
<td>6.90±0.32</td>
<td>3.59±0.12*</td>
</tr>
<tr>
<td>11.</td>
<td>Betamethasone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12.</td>
<td>1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values: Mean ± SEM of six animals *P<0.05  **P<0.01  ***P<0.001
Group 2 compared with Group 1, Group 3 to 8 compared with Group 2.
(b) Effect on serum, liver and granulation tissue protein content in granuloma pouch bearing (GP) rats.

The data have been summarised in Table-21.

**Serum protein content**

Formation of granuloma pouch through carrageenin injection did not alter serum protein content significantly. Significant increase in serum protein content was observed in BET treated rats in comparison to granuloma pouch bearing control rats. ME of both the plants, PBZ and IND did not affect serum protein content.

**Liver protein content**

Liver protein content in GP rats did not differ significantly from normal control rats. Significant increase in liver protein content, in comparison to GP control, was observed in both extract and reference standard treated groups.

**Protein content in granulation tissue**

Neither the test extracts nor reference standards could influence protein content in the granulation tissue significantly.

**TABLE - 21**

EFFECT OF VITEX ALTISSIMA AND VITEX NEGUNDO EXTRACTS ON PROTEIN CONTENT IN SERUM, LIVER AND GRANULATION TISSUE IN GRANULOMA POUCH RATS.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Serum g/dl</th>
<th>Liver mg/g wet tissue</th>
<th>Granulation tissue mg/g wet tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Normal control</td>
<td>5.95±0.37</td>
<td>3.09±0.19</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td>G.P. Control Vitex altissima</td>
<td>5.78±0.41</td>
<td>3.62±0.11</td>
<td>2.12±0.18</td>
</tr>
<tr>
<td>3.</td>
<td>100</td>
<td>5.47±0.20</td>
<td>4.09±0.09**</td>
<td>2.22±0.14</td>
</tr>
<tr>
<td>4.</td>
<td>200</td>
<td>5.63±0.10</td>
<td>4.50±0.13***</td>
<td>2.60±0.18</td>
</tr>
<tr>
<td>5.</td>
<td>200</td>
<td>5.90±0.09</td>
<td>4.87±0.17***</td>
<td>1.58±0.28</td>
</tr>
<tr>
<td>6.</td>
<td>400</td>
<td>5.06±0.09</td>
<td>5.06±0.09***</td>
<td>1.63±0.21</td>
</tr>
<tr>
<td>7.</td>
<td>Phenylbutazone</td>
<td>5.49±0.22</td>
<td>4.64±0.14***</td>
<td>1.86±0.21</td>
</tr>
<tr>
<td>8.</td>
<td>Indomethacin</td>
<td>6.54±0.44</td>
<td>5.40±0.23***</td>
<td>2.26±0.38</td>
</tr>
<tr>
<td>9.</td>
<td>Betamethasone</td>
<td>7.34±0.35*</td>
<td>5.64±0.25***</td>
<td>2.70±0.23</td>
</tr>
</tbody>
</table>

Values: Mean ± SEM

* P < 0.05   ** P < 0.01   *** P < 0.001

Group 3 to 9 compared to Group 2.
TRANSAMINASE ACTIVITY :

Effect on Glutamic oxaloacetic transaminase (GOT) activity in cotton pellet implanted rats:

(a) Serum GOT activity: As could be observed from the data presented in Table-22, statistically non-significant decrease in serum GOT activity was observed in cotton pellet (CP) implanted rats in comparison to normal control rats. In ME of VAL treated rats statistically non-significant increase GOT activity was observed. The decrease in GOT activity observed with ME of VNDL was also statistically non-significant. PBZ had no effect while BET produced significant elevation in serum GOT activity.

(b) Liver GOT activity: Cotton pellet implantation caused significant elevation in liver GOT activity. This elevation was significantly antagonised by pretreatment with both test extracts and the reference standard drugs.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment mg/kg-1</th>
<th>Serum μ mole pyruvate/min litre at 37°C</th>
<th>Liver n mole pyruvate/min/mg protein at 37°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Normal control</td>
<td>80.17±05.42</td>
<td>910.00±110.00</td>
</tr>
<tr>
<td>2.</td>
<td>C.P. Control</td>
<td>55.24±10.12</td>
<td>2160.00±240.00***</td>
</tr>
<tr>
<td>3.</td>
<td>Vitex altissima</td>
<td>70.61±02.78</td>
<td>1421.67±031.13**</td>
</tr>
<tr>
<td>4.</td>
<td>100</td>
<td>78.25±02.74</td>
<td>1038.00±106.08***</td>
</tr>
<tr>
<td>5.</td>
<td>Vitex negundo</td>
<td>44.06±02.77</td>
<td>915.33±019.57***</td>
</tr>
<tr>
<td>6.</td>
<td>200</td>
<td>37.92±02.75</td>
<td>1005.33±125.83***</td>
</tr>
<tr>
<td>7.</td>
<td>Phenybutazone</td>
<td>50.37±01.90</td>
<td>1250.00±200.00*</td>
</tr>
<tr>
<td>8.</td>
<td>100</td>
<td>101.94±07.16**</td>
<td>943.00±40.00***</td>
</tr>
</tbody>
</table>

Data: Mean ± SEM

* P < 0.05  ** P < 0.01  *** P < 0.001
Group 2 compared to Group 1, Group 3 to 8 compared to Group 2.
GOT activity in granuloma pouch bearing rats:

The data have been summarised in Table -23.

(a) **Serum GOT activity**: No significant difference in serum GOT activity could be observed between normal control and GP rats. Significant decrease in serum GOT activity was observed in lower dose (100 mgkg-1) ME of VAL, higher dose (400 mgkg-1) ME of VNDL and BET administered groups. The GOT activity remained unaffected in other extract and reference standard groups.

(b) **Liver GOT activity**: Liver GOT activity did not increase in GP rats in comparison to normal rats and it remained unaffected even after pre-treatment with test extracts and reference standards.

(c) **GOT activity in granulation tissue**: Significant decrease in GOT activity in granulation tissue was observed in ME of VNDL and reference standard administered groups in comparison to GP control rats. ME of VAL did not produce any significant effect on GOT activity in granulation tissue.

**TABLE -23**

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Serum&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Liver&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Granulation tissue&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Normal control</td>
<td>80.17±05.42</td>
<td>913.33±114.06</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td>G.P. Control</td>
<td>83.06±04.23</td>
<td>940.50±113.89</td>
<td>488.33±28.92</td>
</tr>
<tr>
<td>3.</td>
<td>100 Vitex altissima</td>
<td>63.87±03.86&lt;sup&gt;**&lt;/sup&gt;</td>
<td>1111.67±076.22</td>
<td>472.00±84.46</td>
</tr>
<tr>
<td>4.</td>
<td>200 Vitex negundo</td>
<td>86.84±10.80</td>
<td>966.60±56.01</td>
<td>378.33±66.60</td>
</tr>
<tr>
<td>5.</td>
<td>200 Phenylbutazone</td>
<td>75.09±6.24</td>
<td>1023.33±68.05</td>
<td>206.00±50.46&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>6.</td>
<td>200 Indomethacin</td>
<td>45.47±4.34&lt;sup&gt;***&lt;/sup&gt;</td>
<td>1005.00±38.88</td>
<td>330.00±49.60&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>7.</td>
<td>100 Betamethasone</td>
<td>68.93±5.73</td>
<td>866.00±41.55</td>
<td>232.50±52.18&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>8.</td>
<td>5</td>
<td>78.28±3.09</td>
<td>758.00±82.43</td>
<td>272.00±43.98&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>9.</td>
<td>1</td>
<td>57.60±3.35&lt;sup&gt;**&lt;/sup&gt;</td>
<td>813.33±47.80</td>
<td>228.00±36.11&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Data : Mean ± SEM

* P < 0.05    ** P < 0.01    *** P < 0.001

<sup>a</sup>= μ mole pyruvate/min/litre at 37°C   <sup>b</sup>= μ mole pyruvate/min/mg protein at 37°C
Effect on Glutamic pyruvic transaminase
(GPT) activity in cotton pellet implanted (CP) rats:

The data on the effect of test extracts and reference standards on GPT activity in serum and liver homogenate in CP rats have been presented in Table -24.

**Serum GPT activity**: Significant elevation of GPT activity was observed in CP rats in comparison to normal control rats. This elevation in GPT activity was antagonised by pretreatment with ME of VAL and PBZ. ME of VNDL had no effect while increased GPT activity was observed in BET administered group.

**Liver GPT activity**: Elevation of GPT activity was observed in liver homogenate of CP rats in comparison to normal rats. Statistically significant decrease in liver GPT activity was observed in ME of VAL at both the dose levels. Though decrease was also observed in ME of VNDL and reference standards administered groups it was not statistically significant.

**TABLE -24**

**EFFECT OF VITEX ALTISSIMA AND VITEX NEGUNDO LEAF EXTRACTS ON GPT ACTIVITY IN SERUM AND LIVER OF COTTON PELLET IMPLANTED RATS.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment mg/kg⁻¹</th>
<th>Serum a</th>
<th>Liver b</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Normal control</td>
<td>21.60±5.03</td>
<td>1223.33±110.59</td>
</tr>
<tr>
<td>2.</td>
<td>C.P. Control</td>
<td>35.36±3.06*</td>
<td>2667.50±475.68*</td>
</tr>
<tr>
<td>3.</td>
<td>Vitex altissima</td>
<td>100</td>
<td>22.19±1.06**</td>
</tr>
<tr>
<td>4.</td>
<td></td>
<td>200</td>
<td>20.54±2.42**</td>
</tr>
<tr>
<td>5.</td>
<td>Phenylbutazone</td>
<td>200</td>
<td>26.43±3.25</td>
</tr>
<tr>
<td>6.</td>
<td></td>
<td>400</td>
<td>33.83±3.46</td>
</tr>
<tr>
<td>7.</td>
<td>Betamethasone</td>
<td>100</td>
<td>17.57±4.74*</td>
</tr>
<tr>
<td>8.</td>
<td></td>
<td>1</td>
<td>68.31±7.18**</td>
</tr>
</tbody>
</table>

* P < 0.05  ** P < 0.01  Data: Mean ± SEM of six animals
Group 1 compared to Group 2, Group 3 to 8 compared to Group 2.
a = µ mole/pyurate/min/litre at 37°C
b = nmole pyurate/min/mg protein at 37°C
GPT activity in granuloma pouch (GP) rats:
The data on the effect of test extracts and reference standards on GPT activity in serum, liver and granulation tissue homogenate have been presented in Table -25.

Serum GPT activity: Serum GPT activity in GP rats did not differ significantly from GPT activity observed in normal rats. Increase in serum GPT activity was observed in lower dose (100 mgkg-1) ME of VAL administered group, no effect could be observed with higher dose of the extract. GPT activity in ME of VNDL and reference standard treated groups did not differ significantly from GPT activity observed in GP control rats.

Liver GPT activity: GPT activity in liver homogenate of GP rats was significantly less in comparison to normal control rats. Pretreatment with ME of VAL and VNDL caused significant increase in liver GPT activity in comparison to GP control rats. Liver GPT was not significantly affected in reference standard administered groups.

GPT activity in granulation tissue: Significant decrease in GPT activity in granulation tissue homogenates was observed in lower dose (200 mgkg-1) ME of VNDL, IND and BET administered groups in comparison to GP rats. Decrease observed in PBZ treated group was not statistically significant. ME of VAL did not affect GPT activity in granulation tissue significantly.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Serum^a</th>
<th>Liver^b</th>
<th>Granulation tissue^b</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Normal control</td>
<td>21.60±5.03</td>
<td>1223.33±110.59</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td>G.P. Control V. altissima</td>
<td>19.68±6.69</td>
<td>865.00±77.66*</td>
<td>125.64±28.36</td>
</tr>
<tr>
<td>3.</td>
<td>100 V. negundo</td>
<td>36.25±1.91*</td>
<td>1533.53±72.10***</td>
<td>166.75±39.68</td>
</tr>
<tr>
<td>4.</td>
<td>200 V. negundo</td>
<td>17.57±5.29</td>
<td>1303.33±53.15**</td>
<td>378.40±0.134</td>
</tr>
<tr>
<td>5.</td>
<td>400 Phenylbutazone</td>
<td>34.12±3.90</td>
<td>1335.00±38.97**</td>
<td>Traces</td>
</tr>
<tr>
<td>6.</td>
<td>500 Indomethacin</td>
<td>22.40±3.46</td>
<td>1185.00±36.98*</td>
<td>143.30±34.49</td>
</tr>
<tr>
<td>7.</td>
<td>100 Betamethasone</td>
<td>21.79±1.61</td>
<td>1126.60±112.41</td>
<td>69.20±38.02</td>
</tr>
<tr>
<td>8.</td>
<td>5</td>
<td>38.57±8.14</td>
<td>924.00±61.29</td>
<td>Traces</td>
</tr>
<tr>
<td>9.</td>
<td>1</td>
<td>28.64±3.76</td>
<td>1138.33±60.80</td>
<td>Traces</td>
</tr>
</tbody>
</table>

Data : Mean ± SEM of six animals
* P < 0.05   ** P < 0.01   *** P < 0.001
a= µ mole pyruvate/min/litre at 37°C  b = n mole pyruvate/min/mg protein at 37°C
ACID PHOSPHATASE ACTIVITY:

Effect of extracts on acid phosphatase (ACPase activity in cotton pellet implanted rats:

The data have been summarised in Table -26.

(a) Serum ACPase activity: Significant elevation in ACPase activity was seen in cotton pellet implanted rats in comparison to normal control rats. ME of both the plants significantly decreased ACPase activity. PBZ had no effect and the decrease in ACPase activity observed in BET group was not statistically significant.

(b) Liver ACPase activity: Significant decrease in ACPase activity was observed in cotton pellet implanted rats in comparison to normal control rats. ME of VAL decreased ACPase activity in comparison to cotton pellet implanted control rats. Elevation of ACPase activity was observed in ME of VNDL and BET administered groups. The increase in ACPase activity observed in PBZ treated group was not statistically significant.

TABLE - 26

EFFECT OF VITEX ALTISSIMA AND VITEX NEGUNDO LEAF EXTRACTS ON ACID PHOSPHATASE ACTIVITY IN SERUM AND LIVER OF COTTON PELLET IMPLANTED RATS.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Serum a</th>
<th>Liver b</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Normal control</td>
<td>159.00±36.22</td>
<td>70.19±09.24</td>
</tr>
<tr>
<td>2.</td>
<td>C.P. Control</td>
<td>490.00±28.00***</td>
<td>25.13±03.21***</td>
</tr>
<tr>
<td></td>
<td>Vitex altissima</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>100</td>
<td>170.00±70.00**</td>
<td>10.67±02.60**</td>
</tr>
<tr>
<td>4.</td>
<td>200</td>
<td>200.00±60.00**</td>
<td>06.67±01.40***</td>
</tr>
<tr>
<td></td>
<td>Vitex negundo</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>200</td>
<td>40.00±10.00***</td>
<td>108.31±15.22***</td>
</tr>
<tr>
<td>6.</td>
<td>400</td>
<td>124.00±30.00***</td>
<td>102.10±13.03***</td>
</tr>
<tr>
<td></td>
<td>Phenylbutazone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>100</td>
<td>640.00±60.00</td>
<td>077.80±30.14</td>
</tr>
<tr>
<td></td>
<td>Betamethasone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.</td>
<td>1</td>
<td>247.00±111.31</td>
<td>054.54±10.62*</td>
</tr>
</tbody>
</table>

Data: Mean ± SEM
* P < 0.05   ** P < 0.01   *** P < 0.001
Group 2 compared with Group 1, Group 3 to 8 compared to Group 2.
a = n mole phenol released/min/litre at 37°C
b = n mole phenol released/min/mg protein at 37°C
Effect of extracts on acid phosphatase (ACPase) activity in granuloma pouch (GP) rats

The data on the effect of test extracts and reference standard drugs on ACPase activity in serum, liver and granulation tissue have been recorded in Table -27.

(a) Serum ACPase activity: Statistically non-significant increase in ACPase activity was observed in GP rats in comparison to normal control rats. ME of VAL did not produce any significant affect on serum ACPase. Marked decrease in ACPase activity was observed in ME of VNDL, PBZ and BET administered rats. In IND treated rats, statistically non-significant increase was observed.

(b) Liver ACPase activity: ACPase activity did not differ significantly from normal control rats. Decrease in ACPase activity was observed in PBZ and IND treated rats. No significant change could be observed in test extracts and BET treated rats.

(c) ACPase activity in granulation tissue: Significant increase in ACPase activity in granulation tissue was observed in higher dose (200 mgkg⁻¹) ME of VAL and IND treated groups. No significant change could be observed in other groups.

TABLE - 27

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Serumᵃ</th>
<th>Liverᵇ</th>
<th>Granulation tissueᵇ</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Normal control</td>
<td>159.00±036.22</td>
<td>70.19±09.24</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td>G.P. Control</td>
<td>236.00±036.30</td>
<td>86.82±07.78</td>
<td>09.50±01.67</td>
</tr>
<tr>
<td>3.</td>
<td>Vitex altissima</td>
<td>290.00±091.00</td>
<td>90.88±09.02</td>
<td>14.84±04.52</td>
</tr>
<tr>
<td>4.</td>
<td>100</td>
<td>170.00±064.97</td>
<td>90.08±10.42</td>
<td>24.37±02.75***</td>
</tr>
<tr>
<td>5.</td>
<td>200</td>
<td>086.67±003.37**</td>
<td>77.41±10.02</td>
<td>13.77±03.48</td>
</tr>
<tr>
<td>6.</td>
<td>400</td>
<td>Traces</td>
<td>67.76±08.48</td>
<td>17.63±05.22</td>
</tr>
<tr>
<td>7.</td>
<td>Phenylbutazone</td>
<td>080.00±023.09**</td>
<td>39.20±10.30**</td>
<td>15.16±04.69</td>
</tr>
<tr>
<td>8.</td>
<td>Indomethacin</td>
<td>505.00±165.60</td>
<td>40.19±03.81***</td>
<td>52.72±18.94*</td>
</tr>
<tr>
<td>9.</td>
<td>Betamethasone</td>
<td>060.10±011.55***</td>
<td>59.55±10.36</td>
<td>16.81±3.73</td>
</tr>
</tbody>
</table>

Data: Mean ± SEM of six animals

* P < 0.05  ** P < 0.01  *** P < 0.001

ᵃ = n mole phenol released/min/litre at 37°C
ᵇ = n mole phenol released/min/mg protein at 37°C

Group 2 compared with Group 1, Group 3 to 9 compared with Group 2
EFFECT ON OROSOMUCOID LEVEL IN SERUM

(a) In cotton pellet implanted (CP) rats: Significant increase in serum orosomucoid level was observed in CP rats in comparison to normal control rats. Though decrease was observed in test extract administered groups, it was not statistically significant. PBZ had no influence on serum orosomucoid level in CP rats. In BET administered group, statistically non significant decrease was observed. The data have been presented in Table - 28.

**TABLE - 28**

EFFECT OF *VITEX ALTISSIMA* AND *VITEX NEGUNDO* LEAF EXTRACTS ON SERUM OROSOMUCOID LEVEL IN COTTON PELLET IMPLANTED RATS

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Serum orosomucoid level (g/litre)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>0.59±0.09</td>
</tr>
<tr>
<td>C.P. Control</td>
<td>1.23±0.20*</td>
</tr>
<tr>
<td><em>Vitex altissima</em></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>0.96±0.06</td>
</tr>
<tr>
<td>200</td>
<td>0.93±0.10</td>
</tr>
<tr>
<td><em>Vitex negundo</em></td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>0.88±0.06</td>
</tr>
<tr>
<td>400</td>
<td>0.92±0.07</td>
</tr>
<tr>
<td>Phenylbutazone</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>1.15±0.07</td>
</tr>
<tr>
<td>Betamethasone</td>
<td>0.99±0.09</td>
</tr>
</tbody>
</table>

Data: Mean± SEM of six animals
* P < 0.01

(b) In granuloma pouch (GP) bearing rats: The data on the effect of extracts and reference standard drugs on serum orosomucoid level in GP rats have been shown in Fig-28. As could be observed, significant elevation in serum orosomucoid level was noted in GP rats in comparison to normal control rats. Decrease in serum orosomucoid level was observed in test extracts and BET administered groups. However, the decrease was statistically significant only in lower dose (100 mgkg⁻¹) ME of VAL administered group. PBZ and IND had no marked effect.
Serum orosomucoid level (g/L)

NOR - CON - 0.80±0.13, GP - CON - 2.14±0.37, VAL 100 - 1.035±0.15, VAL 200 - 1.61±0.42, VNDL 200 - 1.59±0.20, VNDL 400 - 1.92±0.22, PBZ 100 - 2.44±0.20, IND 5 - 2.33±0.21, BET 1 - 1.91±0.28

Fig.28 Effect of *Vitex altissima* and *Vitex negundo* leaf extracts on serum orosomucoid level in granuloma pouch rats.

**EFFECT ON SERUM CERULOPLASMIN ACTIVITY** :

Significant increase in serum ceruloplasmin activity was observed in GP rats in comparison to normal control rats. Neither of the test extracts nor the reference standard drugs could significantly modify serum ceruloplasmin activity. The data have been presented in Table -29.
TABLE -29

EFFECT OF VITEX ALTISSIMA AND VITEX NEGUNDO LEAF EXTRACTS ON SERUM CERULOPLASMIN ACTIVITY IN GRANULOMA POUCH RATS

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Optical density units (PPD-Oxidase activity)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Normal Control</td>
<td>0.214±0.012</td>
</tr>
<tr>
<td>2.</td>
<td>G.P. Control</td>
<td>0.287±0.020*</td>
</tr>
<tr>
<td></td>
<td>Vitex altissima</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>100</td>
<td>0.255±0.010</td>
</tr>
<tr>
<td>4.</td>
<td>200</td>
<td>0.254±0.022</td>
</tr>
<tr>
<td></td>
<td>Vitex negundo</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>200</td>
<td>0.258±0.005</td>
</tr>
<tr>
<td>6.</td>
<td>400</td>
<td>0.266±0.008</td>
</tr>
<tr>
<td></td>
<td>Phenylbutazone</td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>100</td>
<td>0.292±0.008</td>
</tr>
<tr>
<td>8.</td>
<td>5</td>
<td>0.271±0.014</td>
</tr>
<tr>
<td>9.</td>
<td>1</td>
<td>0.269±0.004</td>
</tr>
</tbody>
</table>

Data : Mean ± SEM of six animals
* P < 0.01

EFFECT ON CONNECTIVE TISSUE CONSTITUENTS IN GRANULATION TISSUE :

(a) Effect on hydroxyproline content :

The data have been presented in Table -30.
Significant decrease in hydroxyproline content of the granulation tissue was observed in all drug and extract treated groups except PBZ administered group, when the values were presented as absolute values i.e. hydroxyproline content in the total granulation tissue. However, significant decrease was observed only in higher dose (400 mgkg⁻¹) ME of VNDL administered group, when the values were presented as relative values i.e. ug/g of granulation tissue. Increase in relative content was observed in BET treated group.
TABLE - 30

EFFECT OF *VITEX ALTISSIMA* AND *VITEX NEGUNDO* LEAF EXTRACTS ON HYDROXYPROLINE CONTENT OF THE GRANULATION TISSUE IN CARRAGEENIN INDUCED GRANULOMA POUCH

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Hydroxyproline content in g. tissue</th>
<th>Absolute</th>
<th>Relative</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>µg/total tissue</td>
<td>µg/g tissue</td>
</tr>
<tr>
<td>G.P. Control</td>
<td>739.67±14.20</td>
<td>39.11±6.56</td>
<td></td>
</tr>
<tr>
<td><em>Vitex altissima</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>200.28±61.58***</td>
<td>32.79±3.36</td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>210.71±85.79***</td>
<td>36.57±4.50</td>
<td></td>
</tr>
<tr>
<td><em>Vitex negundo</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>285.33±57.38***</td>
<td>27.40±5.99</td>
<td></td>
</tr>
<tr>
<td>400</td>
<td>246.81±89.99***</td>
<td>17.95±3.18*</td>
<td></td>
</tr>
<tr>
<td>Phenylbutazone</td>
<td>854.66±158.21</td>
<td>57.31±9.42</td>
<td></td>
</tr>
<tr>
<td>Indomethacin</td>
<td>391.10±87.21**</td>
<td>42.88±5.99</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>391.10±87.21**</td>
<td>42.88±5.99</td>
<td></td>
</tr>
<tr>
<td>Betamethasone</td>
<td>389.44±111.99*</td>
<td>67.53±12.54*</td>
<td></td>
</tr>
</tbody>
</table>

Data: Mean ± SEM of six samples
* P < 0.05    ** P < 0.01    *** P < 0.001

(b) Effect on hexosamine content:

The data have been presented in Table -31. Significant decrease in hexosamine content was observed in lower dose of ME of VAL treated groups when the values were presented as absolute values. Decrease was also observed with higher dose of ME of VAL but it was not statistically significant. No significant change could be observed in other groups. When the data were represented as relative values, ME of neither of the plants could affect hexosamine content in granulation tissue. Increase was observed with IND and BET. PBZ had no effect.
TABLE - 31

EFFECT OF VITEX ALTISSIMA AND VITEX NEGUNDO LEAF EXTRACTS ON HEXOSAMINE CONTENT OF THE GRANULATION TISSUE IN CARRAGEENIN INDUCED GRANULOMA POUCH RATS

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total Hexosamine content</th>
<th>Relative</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Absolute</td>
<td>µg/total tissue</td>
</tr>
<tr>
<td>G.P. Control</td>
<td>17.01±2.85</td>
<td>0.914±0.14</td>
</tr>
<tr>
<td>Vitex altissima</td>
<td>100 0.725±2.5*</td>
<td>1.02±0.14</td>
</tr>
<tr>
<td></td>
<td>200 0.87±3.38</td>
<td>1.22±0.15</td>
</tr>
<tr>
<td>Vitex negundo</td>
<td>200 13.48±1.77</td>
<td>1.17±0.07</td>
</tr>
<tr>
<td></td>
<td>400 11.10±0.98</td>
<td>1.00±0.08</td>
</tr>
<tr>
<td>Phenylbutazone</td>
<td>100 12.97±0.70</td>
<td>0.87±0.05</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>5 13.04±1.40</td>
<td>1.49±0.11**</td>
</tr>
<tr>
<td>Betamethasone</td>
<td>1 10.92±2.24</td>
<td>1.76±0.08**</td>
</tr>
</tbody>
</table>

Data: Mean ± SEM of six samples
* P < 0.05   ** P < 0.01

EFFECT ON RNA AND DNA CONTENT OF THE GRANULATION TISSUE:

The data have been summarised in Table - 32

RNA content: Marked decrease in RNA content was observed in both test extract and reference standard administered groups when the data were presented as absolute values. However, when the data were presented as relative values, the decrease observed was statistically significant only in ME of VNDL, BET and IND administered groups.

DNA content: Marked decrease was also observed in DNA content of granulation tissue in all the drug and test extract given groups when the data were presented as absolute values. However, significant decrease was observed only in PBZ and lower dose ME of VNDL treated groups when the data were presented as relative values.
TABLE -32

EFFECT OF *VITEX ALTISSIMA* AND *VITEX NEGUNDO* LEAF EXTRACTS ON RNA AND DNA CONTENT OF GRANULATION TISSUE IN GRANULOMA POUCH

<table>
<thead>
<tr>
<th>Treatment</th>
<th>RNA</th>
<th>DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Absolute (mg)</td>
<td>Relative mg/g tissue</td>
</tr>
<tr>
<td>Control</td>
<td>245.25±26.88</td>
<td>14.02±2.48</td>
</tr>
<tr>
<td><em>Vitex altissima</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>62.41±19.70***</td>
<td>10.72±1.51</td>
</tr>
<tr>
<td>200</td>
<td>75.36±28.32**</td>
<td>11.98±0.88</td>
</tr>
<tr>
<td><em>Vitex negundo</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>14.99±4.45***</td>
<td>1.21±0.15***</td>
</tr>
<tr>
<td>400</td>
<td>21.12±2.61***</td>
<td>1.89±0.21***</td>
</tr>
<tr>
<td><em>Betamethasone</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>31.51±7.51***</td>
<td>5.05±0.20**</td>
</tr>
<tr>
<td><em>Indomethacin</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>39.52±7.42***</td>
<td>4.44±0.73**</td>
</tr>
<tr>
<td><em>Phenylbutazone</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>164.27±11.05*</td>
<td>11.00±0.37</td>
</tr>
</tbody>
</table>

Data: Mean ± SEM

* P< 0.05  ** P < 0.01  *** P < 0.001
DISCUSSION

Acute toxicity:

The results of the present study show that methanol extract (ME) of *Vitex negundo* leaf (VNDL) to be quite inert since it did not produce any mortality up to the dose of 6000 mg kg^{-1} in mice and 4000 mg kg^{-1} in rats. ME of *Vitex altissima* (VAL) has a high LD_{50} value -(5800 mg kg^{-1} in mice) indicating that it is only slightly toxic. The difference between the two plants with respect to the magnitude of toxicity seems to be due to quantitative differences in their phytochemical composition. Qualitatively, both plants exhibit more or less similar chemical profiles. The wide difference between the effective anti-inflammatory doses and doses producing death indicate that the plants have wide margin of safety.

ANTI-INFLAMMATORY ACTIVITY

Ever since the synthesis of acetylsalicylic acid in 1899 by the German company Bayer and its immediate and successful introduction to clinical practice to treat inflammatory disorders and later on introduction of similar anti-inflammatory drugs, the attention of pharmacologists throughout the world has been focused on elucidation of their mechanisms of action. This is not surprising since inflammatory disorders like rheumatoid arthritis (RA) have world wide prevalence, occur in all races and ethnic groups and have onset in early adulthood some times crippling the afflicted person to render him economically non productive (54, 47).

Attempts to elucidate the mechanism of action of these drugs showed that they inhibit a wide variety of reactions. However, no clear cut relationships could be established between these effects and anti-inflammatory activity (284). In the earlier days many explanations were offered to explain the mechanism of anti-inflammatory effect. Important among them were the theories that the anti-inflammatory activity is due to uncoupling of oxidative phosphorylation (285) and modulation of c-AMP system (286). It was also hypothesised that the vascular events associated with inflammation are mediated by chemical mediators and anti-inflammatory activity may be produced by modulation of their activity. Early studies emphasized the importance of vasoactive amines like histamine and serotonin. Later on prostaglandin and kinins were added to the list (129, 121). Along with this, importance of tissue digestive enzymes released by inflammatory cells such as polymorphonuclear leukocytes, macrophages and eosinophils in causing inflammation was discovered (109, 125). During the next phase number of arachidonic acid metabolites like prostacyclin, thromboxane, leukotrienes etc. were identified and studies were undertaken to assess their role in inflammation (109, 131). In the recent period number of studies (173, 287) have shown that biologically derived oxidants like superoxide anion (O_{2}^-), hydrogen peroxide (H_{2}O_{2}), hypochlorous acid (HOCl) and peroxidase generated oxidants play major role in the tissue injury that results as a consequence of inflammatory response (164). Currently it is widely accepted that the NSAIDs
produce antiinflammatory activity generally, but not exclusively, through inhibition of prostaglandin synthesis. In 1971 Vane (148) showed that all NSAIDs tested were potent inhibitors of prostaglandin synthesis. He proposed that since it is the only common action that was shared by a wide variety of chemical agents, it may be responsible for the anti-inflammatory activity noted with these agents. This hypothesis was further supported by the subsequent studies which showed that prostaglandins are released whenever there is injury to the cell (118) and they appear in inflammatory exudates (118, 288). Further, all the NSAIDs reported till date are capable of inhibiting prostaglandin synthesis in vitro at concentrations that are readily achieved in vivo (289), the rank order of IC50 for inhibitions of PG synthesis corresponds well to the rank order of their clinical effectiveness as antiinflammatory agents and NSAIDs share some common side effects like injury to gastric mucosa (289). However, there is also a view that the above concept may be incorrect (284) because classical prostaglandins are not effective in causing inflammation of appreciable magnitude, though they can cause vasodilation and potentiate the inflammatory effects of other mediators like histamine and bradykinin (118, 131). It has also been reported that PGE injection substantially reduces inflammation in rats with adjuvant arthritis (290). NSAIDs do not generally inhibit the formation of leukotrienes which also contribute to inflammation and they donot modulate synthesis of other mediators of inflammation. These reports focus on the fact that inflammation is a multifactor mediated process and several mechanisms operate during its induction.

It seems likely that plasma proteins, vasoactive amines, tissue digestive enzymes, biologically derived oxidants, eicosanoids all participate in the process of inflammation (291). In addition many other factors like lymphokines are involved in the pathogenesis of inflammation of immunological origins (109, 162).

Since inflammation is a very complex and dynamic process involving series of phase to phase events occurring in an orderly sequence(109), no single testing procedure could be expected to provide complete details about the mechanism of action of anti-inflammatory agents. This has necessitated the development of numerous testing procedures. The main difficulty in designing suitable experimental models is the lack of complete understanding of the aetiological factors responsible for the onset of different types of connective tissue disorders (55).

The process of inflammation is normally considered to occur in three distinct phases (126). They are 1) Vascular phase : It is the phase of hyperaemia and oedema. It is an acute transient phase characterised by local vasodilation and enhanced capillary permeability. 2) Cellular phase : It is a delayed sub acute phase. Infiltration of affected tissue by blood leukocytes and other phagocytic cells is the main feature of this phase. 3) Phase of repair : It includes synthesis of new tissue and repair of the damaged area.
Many mediators of inflammatory process have been identified. Vasoactive amines like histamine, 5-hydroxytryptamine, bradykinin and prostaglandins have been implicated in the production of early vascular events. Role of mediators in cellular phase has not been clearly elucidated as yet, though many substances are reported to possess chemotactic and chemokinetic effects on inflammatory cells (174,151). Leukotriene $B_4$ is reported to be one of the most potent chemotactic agents. Other factors possessing potent chemotactic activity are platelet activating factor (PAF), factor released during activation of complement like C5a and 15-hydroxyeicosatetraenoic acids (HETE) (174,126). Besides these number of poorly defined large molecules have been reported to possess chemotactic activity (174,176). The repair phase includes synthesis of new connective tissue and repair of the damaged area. Role of mediators in this phase is yet to be understood (109).

The experimental models of inflammation used in the present study were selected on the basis of above mentioned factors to assess the anti-inflammatory activity of the extracts as comprehensively as possible. Basically the models were chosen to represent the abovementioned three phases. Besides, the extracts were evaluated in other test models to obtain additional data on the mechanism of action. This included for example, studies on Freund's adjuvant-induced arthritis, hyposaline-induced lysis of human red blood cells (HRBC), castor oil induced diarrhoea and hydrogen peroxide-induced rat RBC lysis. Effect of extracts was also studied on certain biochemical parameters which are supposed to be altered during inflammation.

VASCULAR PHASE

1 Acute oedema: Experimental models selected to represent oedema and hyperaemia were carrageenin-induced hind paw oedema in rats which also served as primary screening method for anti-inflammatory activity and paw oedema induced with histamine, 5-hydroxytryptamine, bradykinin and prostaglandin E2. These served as models to study the effect of test extracts on individual mediators of inflammation.

Carrageenin-induced rat paw oedema:

Till the description of carrageenin hind paw oedema assay by Winter and Co workers (241), number of substances were employed in an attempt to produce reproducible and easily measurable degree of soft tissue oedema in experimental animals. Three types of substances were employed: 1) irritants like formaldehyde, 2) substances which produce oedema through release of vasoactive amines (eg. dextran, egg albumin) and 3) vasoactive amines i.e. histamine and serotonin. But these methods failed to provide a reliable assay. Carrageenin, on the other hand, produced oedema of easily measurable and reproducible magnitude (292) and this response was inhibited by NSAIDs. The main reason for the wide acceptance of this substance as a phlogistic agent is that the activity and potency characteristics of most NSAIDs...
Mechanism of induction of carrageenin oedema has been extensively investigated (121,293). It is a representative model for exudative phase of inflammation. The oedema develops in a biphasic manner. The initial phase occurs within an hour after injection of the phlogistic agent followed by the second phase which persists for a longer duration. The first phase is reported to be due to the release of histamine and 5-hydroxytryptamine. Trauma of injection may also contribute to its development (293). Bradykinins and prostaglandins, especially the latter, are reported to play major role in the development of second phase of oedema. Presence of complement is reported to be required for the optimum development of oedema (293). Bonta and co-workers (294) have reported that prostaglandin release could be detected as early as 1h after carrageenin injection.

The results of the experiment undertaken to assess the effect of extracts on carrageenin paw oedema in rats show that ME of both VAL and VNDL possess significant oedema suppressing activity. Comparison of the ED50 values shows that ME of VAL possess comparatively stronger anti-inflammatory activity. The results confirm and corroborate presence of anti-inflammatory activity in Vitex negundo leaves and reveal presence of significant anti-inflammatory activity in Vitex altissima, a closely related species. At the doses around ED50 values the extracts were not effective in inhibiting the first phase of carrageenin oedema, though the oedema formation was suppressed in both the phases at higher doses. This may be indicative of the fact that at lower doses the oedema suppression by the extracts may be through modulation of the activity of inflammatory mediators of the second phase viz. kinins and prostaglandins. At higher doses the effect may be mediated through modulation of mediator activity in both the phases. The exact nature of the extract's modulation effect remains to be elucidated. The oedema suppression may be due to interference with the release of mediators in the affected area or the extracts may be antagonising the activity of the mediators after their release. The extracts failed to produce significant anti-inflammatory effect after oral administration. This indicates that they are not absorbed completely through gastrointestinal tract.

Since the extracts were found to possess significant anti-inflammatory effect in the primary screening tests, it was felt useful to study their effect in oedema induced by other inflammatory mediators. The results of the study showed that ME of both VAL and VNDL produced weak to moderate suppression of histamine-induced hind paw oedema in rats. However, neither of the extracts could antagonise 5-hydroxytryptamine induced rat paw oedema. This result is consistent with the observation noted in carrageenin hind paw oedema in which the extracts, at similar dose level, did not produce significant suppression of initial phase of oedema formation which is reported to be due to the release of histamine and 5-hydroxytryptamine. However, the extract produced significant suppression of...
bradykinin-induced hind paw oedema in rats. Prostaglandin $E_2$ induced paw oedema was significantly antagonised by ME of VNLD and the antagonism observed with ME of VAL was not statistically significant. The results show that ME of VNLD produces its anti-inflammatory activity predominantly through modulation of the activity of inflammatory mediators in the second phase. Since ME of VAL failed to produce significant antagonism of $PGE_2$ induced oedema, additional mechanism(s) may be involved in its anti-inflammatory effect.

It is a well known fact that the mediators produce their effect through activation of receptors, specific to them, present on the cell surface (131). In addition to their individual effects the mediators may interact in a complex manner to produce hyper-responsiveness (131). It has been reported that mediators such as $PGE_2$ and $PGI_2$ which cause increased blood flow, potentiate the plasma exudation caused by other mediators like bradykinin (131). The mediators may also act by 'sensitising' or 'priming' the inflammatory cells (129). Kinins are reported to possess the ability (129) to release cytokines (IL1, TNF) and other mediators like prostaglandin and leukotrienes (129, 131). This may be one of the important mechanism of their phlogistic activity. Receptors have been described for each of the mediators involved. Bradykinin, which is an important inflammatory mediator, is reported to produce its effect through 3 types of receptors (132). BK1, BK2 and BK3, however, some workers (129) have described only two types of receptors. Most of the actions of bradykinin are reported to be mediated through BK2 receptor (129). Seven receptor sub types have been described for prostaglandins (295). PGE's are reported to produce their effect through $EP_1$ & $EP_2$ receptors. It is a well known fact that the mediator action may be produced through activation of second messenger system (146). It is possible that a given drug may modulate the effect of mediator at different sites like receptor site and the site of coupling of receptors to effector mechanisms. The drug may act by preventing mediator interaction and 'priming' of inflammatory cells. In addition steroids act by inhibiting phospholipase $A_2$ leading to decrease in the availability of arachidonic acid which is the precursor fatty acid for the synthesis of different type of eicosanoids (194, 296). This effect is brought about indirectly by induction of synthesis of lipocortin which inhibits phospholipase $A_2$ (291). It would be useful and interesting to assess the extracts for the above mechanisms of action.

2. Vascular permeability:

Increased vascular permeability at the site of injury is another important feature of vascular phase of acute inflammation. Vascular permeability increases in the microcirculation (109) and is mediated through the release of inflammatory mediators. The effect is exerted at small venules and involves separation of junction between the endothelial cells (114). The exact mechanism of the increase in permeability is not clear. It is reported to involve activation of contractile proteins within the cytoplasm of endothelial cells (109).
Increased vascular permeability is normally assessed by measuring the volume of fluid exuding from the injured vessel or by labelling the plasma proteins and determining the amount of label that accumulates in the damaged tissue (297). Effect of test extracts on capillary permeability was assessed by measuring their effect on the extravasation of Evans blue into the oedema tissue following carrageenin injection into the rat paw. ME of both VAL and VNDL produced significant inhibition of carrageenin induced Evans blue extravasation into oedema tissue. This indicates that the extracts possess antagonistic effect on the phlogistic agents induced increased capillary permeability. Since the enhanced vascular permeability is due to release of inflammatory mediators (248), it can be assumed that the noted effect is due to their modulation. The exact nature of modulatory effect of extract on the mediators remains to be elucidated.

The antagonistic effect of extracts on vascular permeability was further corroborated by the results obtained in carrageenin pleurisy and PVC cup implantation tests in rats. ME of VAL produced significant inhibition of fluid exudation in both the tests. ME of VNDL produced marked inhibition of fluid exudation in PVC cup. Inhibition was also observed in carrageenin pleurisy test but it was of lesser magnitude and statistically non-significant. This discrepancy may be due to the crude nature of the extract or due to the inherent differences between the experimental procedures (211). Since, the extract (ME of VNDL) produced significant inhibition in 2 out of 3 models, it can be inferred that the inhibition of vascular permeability is an important component of the anti-inflammatory activity observed in the extracts.

CELLULAR PHASE:

One of the earliest events, irrespective of nature of the inducing agent, accompanying acute inflammation is the pavementing of endothelium by the neutrophils (298). Adherence of cells to the endothelium is a prerequisite for their subsequent diapedesis into the extravascular compartment (109). Neutrophils are the first to be seen in early inflammation, followed by small numbers of monocytes.

Many techniques have been employed by different workers to assess the effect of test drugs on cell emigration (211, 299, 300). In the present study PVC cup implantation method first described by Venkataraman (301) was employed. ME of both VAL and VNDL produced marked inhibition of leukocyte emigration into exudate formed in PVC cup implants. However, the effect on differential count in the exudate did not reveal decrease in neutrophil percentage. Inhibition of leukocyte emigration seems to be one of the mechanisms of anti-inflammatory activity noted with the extract.

Polymorphonuclear leukocytes and macrophages are important for the production of inflammation. They migrate towards the site of injury. They can perceive gradients of chemoattractant molecules (197) and migrate directionally along the gradient. These cells
possess specific surface receptors for chemoattractant agents like C5a, LTB4 and CCF. Binding of these agents to the phagocyte cells causes many changes (174). The cells lose their round configuration and assume triangular shape with base of the triangle facing towards the chemoattractant gradient. This change in shape is brought about by rearrangement of cytoskeletal elements inside the cells. Microtubules provide front to back polarization whereas the contractile force is provided by action filaments which accumulate at the front and back of the cells. These agents stimulates appearance of several adhesive proteins on their surface to facilitate their attachment to vascular endothelium which is necessary for their motility (129). They also enhance the expression of surface receptor for the mediators through translocation from intracellular reservoir (174). The mechanisms through which formation of endothelial gap occurs remains to be elucidated. It is possible that the extracts induced inhibition of leukocyte migration might be mediated through the above mechanisms. However, further studies would be required employing suitable experimental procedures to elucidate the exact mechanism of inhibition.

PHASE OF REPAIR:

This phase is characterised by proliferation of fibroblasts, synthesis of new tissue and repair of the damaged area.

Formaldehyde-induced arthritis in rats is used by number of workers as a representative model for studying drug effect on the proliferative phase of inflammation (211, 248). Significant proliferation of fibroblasts has been reported to occur after formaldehyde injection. ME of both VAL and VNDL produced significant suppression of formaldehyde induced paw oedema. ME of VAL produced comparatively stronger effect. This indicates that inhibition of proliferative phase of inflammation contributes significantly to the anti-inflammatory effect of the extract. The exact nature of the modulators involved in fibroblast proliferation after formaldehyde injection is not known.

Effect on granuloma formation:

The repair phase of the inflammation begins with the proliferation of fibroblast and multiplication of small blood vessels. The cellular proliferation penetrates the exudate producing a highly vascularised reddish mass which is called granulation tissue (302). Granuloma is a chronic inflammatory lesion in the form of a tumour resembling mass (109). It represents both exudative and proliferative phases of inflammation (248). Formation of fibrous tissue predominates over fluid accumulation. The inflammatory mass consists of inflammatory cells, area of granulation and fibrous tissue thus representing intermingling of healing and inflammation which is the main feature of chronic inflammation (109). Healing process which involves formation of connective tissue is modulated by finely tuned interaction
between inhibitory and growth factors. Growth factors are reported to exert their effect by activating the somatic cells from resting phase to phase of synthesis (190). Besides, lymphokines are also reported to increase fibroblast migration and division (303).

Subcutaneous implantation of cotton pellets in rats induces formation of granuloma exhibiting characteristic features of a typical developing chronic inflammatory lesion. One drawback of this technique is that it is not possible to measure exudative fluid component of chronic inflammation. In granuloma pouch model in which granulation tissue formation is induced by injection of irritants, both exudative and proliferative components can be measured. Effect of test extracts was studied on granulation tissue formation in both cotton pellet implanted rats and carrageenin induced granuloma pouch bearing rats. Carrageenin induced granuloma pouch model was preferred to the croton-oil induced granuloma pouch model since the former has certain advantages over the latter model. In carrageenin model necrosis of the pouch wall does not occur and the formed granulation tissue can be easily separated from the body and the exudate fluid can be measured accurately (248). ME of both VAL and VNDL produced marked decrease in granulation tissue formation in cotton pellet implanted rats. In carrageenin induced granuloma bearing rats also the extracts produced significant decrease in granulation tissue formation. ME of VAL suppressed fluid exudate at both the dose levels studied whereas ME of VNDL did so only at higher does level. In this model ME of VAL produced greater effect in comparison to equipotent dose of ME of VNDL.

The results indicate that the extracts exert inhibitory effect on proliferative phase of inflammation also. Inhibition of exudate formation in this model further confirms the presence of fluid exudation inhibitory effect in the extracts. Proliferative phase of inflammation can be modulated through diverse mechanisms. Inhibition of migration of inflammatory cells to the site of injury can be one of the main mechanisms. This can occur either by inhibition of release of chemoattractant factors or through antagonisms of their effect after release. Modulation of events occurring at the receptors coupling to effector system can be another possible mechanism. Inhibition of formation of connective tissue and exertion of cytotoxic effect on the involved cells may also lead to attenuation of proliferative response (304). The extracts inhibit leukocyte emigration. Whether they modulate connective tissue formation and possess cytotoxic effect on the inflammatory cell remains to be assessed.

INFLAMMATION OF IMMUNOLOGICAL ORIGIN

1) Adjuvant-induced arthritis

Efforts for developing effective antirheumatic drugs have been hampered by the lack of appropriate animal model of arthritis (292). Arthritis has been induced in animals by employing different techniques like adjuvant-induced arthritis (292, 305, 306), collagen induced arthritis (307), antigen induced arthritis (308) and evolving genetic model (MRL/lpr
mouse) (309). Among these, adjuvant and collagen induced arthritis have been useful, though their underlying pathophysiological mechanisms have many differences in comparison to human rheumatoid arthritis (292).

Adjuvant arthritis is the most widely used animal model of chronic arthritis (211). It is considered desirable to include this model for predicting clinical efficacy of any new anti-inflammatory drug in rheumatoid arthritis (211). Elicitation of the arthritic syndrome is reported to be due to the peptidoglycans of the cell wall of mycobacteria, which is one of the components of the Freund's complete adjuvant (310). Injection of adjuvant induces classical immune response in which T and B lymphocytes and antigen presenting cells participate. The arthritic syndrome is believed to be due to cell-mediated immunity. It is characterised by swelling of the soft tissue around ankle joints initially which is due to oedema of ligaments and joint capsules of the involved joints. As the disease progresses changes similar to occurring in human rheumatoid arthritis i.e. diffuse demineralisation, osseous erosion and cartilage loss occurs (311). Inflammation of synovial membrane occurs. The synovitis is characterised by leukocyte infiltration and proliferation of fibroblasts in the synovial membrane. In addition deposition of new bone also occurs (311). Cartilage loss takes place with loss of chondrocytes and medullary bone resorption (311). Lymphokines (especially IL1, IL6) secreted by the activated T-cell play important role in the development of this experimental arthritis by causing accumulation and activation of macrophages, fibroblasts and lymphocytes. They enhance and promote proliferation of cartilage cells and chondrocytes (312). They are also reported to be responsible for the enhanced degradation of both proteoglycan and collagen components by activating and releasing collagenase and related enzymes from the chondrocytes (311, 312).

Results of the study undertaken to assess the effect of test extracts on Freund's adjuvant induced arthritis showed that, contrary to the expectations, the extracts failed to suppress primary oedema. While ME of VNDL suppressed secondary oedema ME of VAL did not influence the formation of secondary oedema. In this test phenylbutazone (PBZ) was administered in 50mgkg⁻¹ dose (ip) since higher dose (100 mgkg⁻¹) produced mortality. At this dose PBZ also failed to inhibit primary oedema, however it suppressed secondary oedema. Dexamethasone, which was used as second reference standard inhibited both primary and secondary oedema. The results indicate that ME of VAL may not be effective in treatment of RA. ME of VNDL by suppressing secondary oedema indicates that it may have modulatory effect on immunological component of the FA induced arthritis. It is difficult to explain ineffectiveness of the extracts on primary oedema inspite of observing significant anti-inflammatory effect in acute inflammatory models.
2) SRBC Induced paw oedema in mice:

By varying the concentration of SRBC and immunisation schedule both antibody and cell mediated response can be elicited in mice. Single injection of high concentration induces antibody formation. When mice are sensitized with low concentration followed by challenge with similar concentration through injection into the paw, oedema formation occurs which is reported to involve delayed type of hypersensitivity representing cell mediated immunity (CMI). Hence, the extracts were tested in this model of CMI as a model involving second species. The extract administration was started prior to sensitization and continued till the challenge covering all the phase in the development and expression of cell mediated immune response. ME of VAL at 200 mg kg⁻¹ produced significant suppression of oedema at both the time intervals at which the paw volume was measured. The paw oedema suppression observed with ME of VNDL was statistically significant only with regards to paw volume measured at 24h after the challenge with SRBC. Contrary to our expectation, variable results were observed with the two models of CMI employed in this study. ME of VAL through ineffective in Freund's adjuvant induced arthritis, produced significant suppression of SRBC induced immunological oedema in mice. ME of VNDL suppressed secondary oedema in Freund's adjuvant induced arthritis while its effect on SRBC-induced pedal oedema was comparatively weaker. ME of VNDL may prove to be useful in the treatment of inflammation of immunological origin. Two explanation could be offered to explain the ME of VAL's effectiveness in suppressing SRBC paw oedema in mice and its ineffectiveness in Freund's adjuvant-induced arthritis. The difference may be due to inherent difference between the two species of the laboratory animals used. It is also possible that mechanism of induction of CMI in the two models may involve different set of factors. The immunological oedema is induced as a complex interaction among various subsets of T-lymphocytes (313) through the release of soluble factors from them. It would be interesting to study whether the extracts modulate release of these factor or antagonise their effect on target cell after release.

OTHER MECHANISMS OF ANTI-INFLAMMATORY ACTIVITY:

Local Irritation:

Many workers (262, 263) have pointed out that route of administration should not have any influence on the anti-inflammatory activity of a true anti-inflammatory agent. In comparison to this, drugs producing irritation may produce anti-inflammatory activity at one site by producing irritation elsewhere. Such drugs are called counter-irritants (314). The anti-inflammatory activity of counter-irritants may be due to release of acute phase proteins such as α2-macroglobulin and α1-antitrypsin with anti-protease activity (315).

Shahanan and coworkers (262) have reported that counter-irritants can be distinguished from true anti-inflammatory agents by co-administering them with phlogistic agents like carrageenin. True anti-inflammatory agents produce oedema suppression when administered
locally with phlogistic agents. On the other hand counter-irritants potentiate oedema formation when administered locally with phlogistic agent. It has been shown by these authors that all the clinically effective anti-inflammatory agents produce anti-inflammatory activity when administered locally with carrageenin. ME of VAL produced significant suppression of oedema on local co-administration with carrageenin when the paw volume was measured 3h after carrageenin injection. The oedema suppression observed with ME of VNDL was not statistically significant. The results shows that ME of VAL possesses local anti-inflammatory activity and its anti-inflammatory activity is not mediated through counter-irritation. ME of VNDL does not possess significant local anti-inflammatory activity. Since it did not potentiate the oedema formation, counter-irritation does not seem to contribute significantly to its anti-inflammatory activity.

Assessment of role of adrenal glands in the anti-inflammatory activity of the extracts:

Stimulation of adrenal pituitary axis resulting in the release of endogenous corticosteroids may be one of the mechanisms of anti-inflammatory effect in a test drug (265). To assess the role of adrenals in the anti-inflammatory activity of ME of VAL and VNDL, the effect of their administration on weight of adrenal gland and adrenal ascorbic acid was noted. Besides their effect on the cytoarchitecture of adrenal gland and on carrageenin paw oedema in adrenalectomised rats was assessed.

a) Adrenal weight:

Significant gain in adrenal weight was observed in lower dose (200 mgkg⁻¹) ME of VNDL administered group in cotton pellet-implanted rats. ME of VAL did not produce significant effect. In granuloma pouch rats, adrenal weight gain was observed in higher dose (200 mgkg⁻¹) ME of VAL and lower dose (200 mgkg⁻¹) ME of VNDL administered rats.

b) Effect on ascorbic acid content of adrenal gland:

Adrenal ascorbic acid content has been reported to reflect the functional status of the gland. Increased adrenal activity causes depletion of adrenal ascorbic acid content and decreased adrenal activity leads to accumulation of ascorbic acid in the gland. This is the basis for using it as a biochemical marker to assess the adrenal activity. Significant decrease in adrenal ascorbic acid content was observed in extracts treated group in comparison to control cotton pellet implanted rats indicating increased adrenal activity.
c) Histological changes in adrenal gland:

Microscopic examination of adrenal sections from extract treated groups showed features of weak to moderate stimulation. In the light of these observations, it can be inferred that stimulation of adrenal pituitary axis is one of the mechanisms of anti-inflammatory activity of the extracts. To further assess the extent of adrenal involvement in the anti-inflammatory activity, the extracts were evaluated for anti-inflammatory activity in adrenalectomised rats.

d) Anti-Inflammatory effect in adrenalectomised rats:

The results of the study showed that adrenalectomy did not influence significantly the anti-inflammatory activity observed in ME of VNDL. ME of VAL also produced significant anti-inflammatory activity in adrenalectomised rats, but the effect was comparatively less in comparison to the anti-inflammatory effect observed in intact rats. Thus adrenal gland does not seem to play important role in the anti-inflammatory activity of ME of VNDL. Part of the anti-inflammatory of ME of VAL seems to be mediated through stimulation of adrenal-pituitary axis.

Effect on membrane stability:

Polymorphonuclear leucocytes and mononuclear phagocyte systems are mainly responsible for maintaining normal host defence against invading microorganisms and clearing tissue of dead cell and debris (tissue hygiene). Mononuclear phagocytes also participate in wound healing and general homeostatic function (109). Accumulation of these cells at the site of injury is one of the main features of acute inflammation. These cells kill the invading organism and scavenge the dead tissue through the process of phagocytosis. Phagocytosis occurs through the formation of phagolysosomes (316). This occurs by adherence or binding of the material to the cell surface, its engulfment into a vacuole and fusion of lysosome with the vacuole. Fusion of membrane is followed by discharge of lysosomal enzymes and other contents of lysosome granules into the vacuole. This causes destruction of the ingested noxious agents (125). Lysosomal enzymes are capable of degrading complex macromolecules. Leukocytes contain several types of lysosomal granules. Two important types called primary and secondary granules can be differentiated by their staining characteristics. Around 60 types of enzymes are reported to be presented in lysosome (316). These include proteases like collagenases, elastase, cathepsin D, Cathepsin G and gelatinase, glycosidas, nucleases, phosphatases, phospholipases, sulphatase, etc. (125, 316). Proteinases are capable of destroying extracellular structures. Cathepsin D cleaves cartilage proteoglycan, collagenase cleaves type I and III collagen of bone, cartilage and tendon. Elastases destroy collagen crosslinkage and proteoglycan, as well as elastin components in blood vessels, ligaments and cartilages. Lysosomal hydrolases trigger formation of inflammatory mediators through activation of complement, kininogen and 128.
plasmogen. Besides release of lysosomal enzymes, stimulated leukocytes also generate highly reactive oxygen radicals like superoxide anion and singlet oxygen which have powerful tissue destruction potential. Under certain circumstances, especially when the inflammatory stimulus is not removed, the accumulated and active leucocytes release their lysosomal contents extracellularly causing damage to the surrounding tissue (174, 197). Lysosomal content release is one of the important cellular events in the development of inflammation. From the above account it is evident that drug capable of inhibiting release of lysosomal content will have significant anti-inflammatory activity.

The precise nature of the mechanisms involved in release of lysosomal content is yet to be understood. Some of the mechanisms mentioned are incomplete phagolysosomes, reverse endocytosis, cell death and modulation of the function of contractile microfilament system (316). Lymphokines, activated complement factor and immune complexes are reported to degranulate lysosomes through activation of cell surface receptors specific to them (174, 197). The release is also modulated by activation of specific protein kinases by cyclic adenosine 3'-5'-monophosphate (c-AMP) and c-GMP (174). Glucocorticoids and non-steroidal anti-inflammatory agents are reported to enhance the stability of lysosomal and cell membranes. This cell membrane stability enhancement effect is supposed to be one of the important mechanisms of their anti-inflammatory activity. Hence, it was thought necessary to assess the test extracts for enhancement of cell membrane stability.

Two methods one in vivo and one in vitro were employed to assess the membrane stability. The in vivo method used was nystatin induced paw oedema. Hyposaline induced lysis of human red blood cells (HRBC) served as in vitro model. Nystatin is reported to produce paw oedema by labilizing the lysosomal membrane leading to release of protease enzymes which activate different systems involved in the formation and release of inflammatory mediators (254, 245). HRBC membrane is reported to be similar to lysosomal membrane in many respects (268). Hence this in vitro system has been advocated for studying the effect of test drugs on membrane stability (268, 269). ME of both VAL and VNDL produced significant inhibition of nystatin induced rat paw oedema and hyposaline-induced HRBC lysis. In nystatin test, both extracts produced almost similar magnitude of inhibition in equipotent doses. In in vitro tests, ME of VAL produced comparatively stronger inhibition.

On the basis of these results it can be suggested that enhancement of stability of lysosomal membrane may be one of the important mechanisms of anti-inflammatory activity observed with the extracts. It would be interesting to elucidate the exact mechanism of this activity. Several mechanism have been proposed as causes of destabilization of cell membranes (317). Changes in protein or lipoprotein structure of cell membrane and disruption of the phospholipid component of cell membrane through release of inflammatory mediators (PGE2) are two of the important mechanisms. NSAID's are reported to enhance membrane stability through protein component of the cell membrane. Since both the extracts
were effective in *in vitro* system, it seems they have direct effect on cell membrane perhaps through interaction with cell membrane components. It is also possible that the extracts may be acting in *in vivo* system through interference with mediators induced disruption of cell membrane, either through inhibition of their release or through antagonising their effect.

**Effect on mast cell degranulation:**

Mast cells are basophil like cells present in different tissues throughout the body. They are characterised by the presence of oval shaped electron dense granules. They along with basophils play important role in anaphylaxis and inflammation associated with it. They are the target cells for IgE binding in anaphylaxis and allergic phenomena. The union of antigen and IgE on the surface of mast cells and basophils cause degranulation of these cells (191, 192) leading to release of inflammatory mediators like histamine, serotonin, prostaglandins, leukotrienes and platelet activating factor (318), chemotactic factors for neutrophils and eosinophils (318) and tissue damage and repair factors (318). The inflammatory mediators released are responsible for the symptoms associated with anaphylaxis. Besides the immunological mechanism, mast cell degranulation is caused by number of agents (319). These agents cause degranulation mainly through elevation of Ca++ ions inside the cell. Some (eg. mastoparan) cause release by directly stimulating guanidine nucleotide-binding regulatory protein (G protein) (318, 320). Others like polymyxin B, act primarily through mobilisation of Ca++ from cellular stores (318). Since mast cell activity can be modulated by number of factors, drugs possessing mast cell degranulation inhibiting effect will have important place in treating immunological inflammation of anaphylaxis type. NSAIDs have been reported to inhibit the release of vasoactive amines from mast cells induced by variety of stimuli (319). Taking into consideration all these factors, it was thought necessary to assess the effect of extracts on mast cell degranulation.

Effect of extracts was studied on egg albumin (in pre-sensitised rats ) and carbachol induced mast cell degranulation. ME of both VAL and VNDL produced significant inhibition of both carbachol and egg albumin induced degranulation. The results indicate that like NSAID's the test extracts possess mast cell degranulation inhibiting effect. Degranulation is a complex phenomenon involving several steps with varying requirements of energy and several factor can modulate the process at different stages (319). Some of the important stages in degranulation caused by antigen binding to IgE are : Activation of cellular pro-esterase to esterase requiring extracellular calcium. This reaction is further augmented by autocatalytic activity of the released esterase. Next stage is movement of granules alongside microtubules through microfilaments and this requires energy and sequential exocytosis of vasoactive amines occurs. Carbachol-induced degranulation is reported to be mediated through elevation of cellular c-GMP levels (270). NSAIDs are reported to inhibit mast cell degranulation by the following mechanisms. i) Elevation of c-AMP level perhaps through inhibition of phosphodiesterase, the enzyme concerned with degradation of c-AMP ii) By
altering the calcium movement across cell membrane and iii) through influence on the calcium mobilisation within the mast cell. The extracts induced inhibition of egg albumin induced degranulation might be through alterations of the entry of calcium and through inhibition of calcium mobilisation inside it. Since carbachol induces mast cell degranulation through activation of guanylyl cyclase, the extract may be acting by modulating the activity of carbachol on guanylyl cyclase. However, the exact mechanism of action remains to be elucidated through further studies. The results indicate that the extracts due to their mast cell degranulation inhibiting effect may be of potential use in immunologically mediated inflammatory conditions especially in type I reactions.

**Castor oil induced diarrhoea:**

Castor oil is reported to induce diarrhoea through formation of prostaglandins in the intestine (273). Many workers have used it as an indirect model for assessing the test drugs effect on prostaglandin formation (272, 273, 274). ME of both VAL and VNDL produced significant inhibition of castor oil-induced diarrhoea like phenylbutazone, which was used as reference standard. The results indicate that the extracts may have inhibitory effect on prostaglandin formation.

**Effect on hydrogen peroxide-induced rat red blood cell lysis:**

Release of toxic oxygen radicals like superoxides anion (O$_2^-$) from the activated polymorphonuclear leukocytes and monocytes macrophage system is considered to be one of the important mechanisms through which tissue injury is produced during inflammation (321). The superoxide released into extracellular tissue induces generation of other activated free radicals such as hydrogen peroxide, hydroxyl radical (OH) and singlet oxygen (O$_2^\cdot$) which also take part in tissue destruction. Inhibition of generation of superoxide or its scavenging has been postulated to be one of the mechanisms to control inflammation (322). Infact the enzyme superoxide dismutase that scavenges oxygen free radicals has been proposed as one of the endogenous anti-inflammatory factors (222).

In the present study, hydrogen peroxide (H$_2$O$_2$) induced lysis of rat red blood cell has been used as a method for assessing free radical scavenging activity in the test extracts (275). It has been hypothesized that free radicals generated form hydrogen peroxide react with erythrocyte membrane to form lipid peroxides which are presumed to be responsible for the haemolysis by destroying cell membrane structures. Neither of the extracts could inhibit H$_2$O$_2$-induced rat RBC lysis. However, it is interesting to note that in this system, contrary to the reported efficacy of NSAIDs to interfere with free radical generation, the reference standard drugs phenylbutazone and indomethacin showed significant increase in haemolytic activity indicating enhanced free radical generation. This calls for caution in interpreting the results obtained in this assay system. It is necessary to test the extracts in other free radical generating assay systems before arriving at a (222) definitive conclusion.
Drug Interaction studies:

Combined administration of test extracts in sub-effective dose with sub-effective dose of phenylbutazone (PBZ) resulted in the potentiation of the anti-inflammatory activity. This synergistic effect indicates that the extracts may possess mechanism of actions similar to those of PBZ.

Pre-treatment of rats with ascorbic acid failed to enhance the anti-inflammatory activity of the test extracts when administered in sub-effective doses. Ascorbic acid produces anti-inflammatory activity by increasing the capillary resistance. Lack of synergistic activity with ascorbic acid indicates that the extracts may not have significant modulatory effect on capillary resistance. Similar type of results were obtained with adrenaline. This is suggestive of non-involvement of endogenous adrenaline in anti-inflammatory activity of the extracts.

Reserpine pretreatment did not potentiate anti-inflammatory activity of ME of VAL when the extracts were administered in sub-effective dose. However, significant potentiation of anti-inflammatory activity was observed with ME of VNDL when administered to rats after reserpine pretreatment. Reserpine is a potent depletor of endogenous amines in number of organs (323). Catecholamines act as anti-inflammatory factors and 5-hydroxytryptamine is reported to be pro-inflammatory (324). Modulation of endogenous amine release does not seem to be the mechanism of anti-inflammatory activity in the ME of VAL. The synergistic effect of reserpine with ME of VNDL (sub-effective dose) may be due to depletion of 5-hydroxytryptamine.

BIOCHEMICAL STUDIES ON THE ANTI-INFLAMMATORY ACTIVITY OF THE EXTRACTS:

Biochemical studies are considered to be helpful for understanding the changes occurring at cellular level with a given drug. Biochemical studies are undertaken to study and understand various mechanisms underlying the induction and sustenance of inflammatory process. They cover almost all aspects of inflammatory phenomenon like studying the test drugs effect on formation, metabolism and role of mediators in different phases of inflammation. Some of the important areas of active research are formation of cyclooxygenase and lipoxygenase products of arachidonic acid metabolism which are reported to play important role in vascular and cellular phases of inflammation (126, 146). Factors involved in the formation of connective tissue constituents and their organisation within it (325), studying the role of chemotactic factors elaborated by inflammatory cells and the affected tissue (174) and measurement of nucleic acid to assess the cellular content at the site of inflammation (326). Studies to assess test drugs effect on enzymes which are supposed to play important role in energy yielding and metabolism like succinic dehydrogenase, lactic dehydrogenase and
ATPase, (211,327) measurement of activity of enzymes like acid phosphatase, B-glucuronidase which serve as biochemical markers to assess lysosomal membrane integrity, measurement of ascorbic acid content in adrenal gland and serum corticosteroid levels to assess the involvement of adrenal-pituitary axis in anti-inflammatory activity and effect on transaminase activity (326,327) are some of the frequently studied parameters. In recent days increased attention is being focussed on the measurement of acute phase reactants like orosomucoid, ceruloplasmin and α1-antitrypsin after it was reported that their elevation in inflammatory reaction is selectively inhibited by disease modifying anti-rheumatic drugs (328). Number of studies have been undertaken on the role of toxic oxygen radicals in inflammation and their modulation by anti-inflammatory drugs (317). Role of cytokines in the formation of inflammatory lesion in affected joints in rheumatoid arthritis (312) is another area of active investigation.

Different workers have adopted different approach while undertaking biochemical studies on anti-inflammatory activity. The data obtained with regards to some of the parameters are often conflicting in nature (211,329). Correlation between changes noted in the parameters studied and the anti-inflammatory activity has rarely been reported. This is particularly so in case of in vivo experiments. Models of experimental inflammation used also differ from investigator to investigator. Inspite of this, biochemical studies are undertaken as a means of obtaining data to facilitate the understanding of drug action.

In the present study effect of test extracts on protein content, transaminase activity, acid phosphatase activity in serum, and liver in cotton pellet implanted and granuloma bearing rats was studied. These parameters were also studied in granulation tissue obtained from granuloma pouch bearing rats. Other parameters studied were orosomucoid content and ceruloplasmin activity in serum and hydroxyproline, hexosamine, RNA and DNA content of granulation tissue.

Protein content:

Increase in liver protein content is indicative of increase in the enzyme content of the endoplasmic reticulum of the liver. This increases with hepatic enzyme stimulating drugs. Many NSAIDs share this property. Neither cotton pellet-implantation nor granuloma pouch formation could affect liver protein content significantly. Liver protein content increased significantly with extract treated group in granuloma pouch bearing rats. However, in cotton-pellet implanted rats, significant increase was observed only with ME of VAL (at both the dose levels) and lower dose of ME of VNDL administered group. The increase was not significant with higher dose of ME of VNDL. Though this discrepancy is difficult to explain, the result indicate that the extracts, similar to NSAIDs, possess endoplasmic reticulum enzyme stimulating property.
The standard drugs and the extracts did not alter protein content of granulation tissue obtained from granuloma pouch bearing rats. Serum protein level was significantly high in cotton pellet-implanted rats in comparison to normal rats. However, no such rise could be observed in granuloma pouch bearing (GP) rats. Increase in serum protein content is normally reflective of increase in tissue destruction and inflammation (330). Increase noted in the present study may be attributed to elevated GPT activity in CP rats. In GP rats the serum protein content did not increase so also transaminase activity. The difference between the two models with regards to serum protein content may be due to different nature of inflammatory stimuli. The increase in serum protein content observed in CP rats was antagonised by the extract treatment. However, the decrease in GPT activity did not correlate well with changes observed in protein content. Significant decrease in serum GPT activity was observed with ME of VAL while the decrease observed with ME of VNDL was not statistically significant.

Transaminase activity:
Transaminases are among the most frequently studied biochemical parameters. Glutamic pyruvate transaminase (GPT) and Glutamic oxaloacetate transaminase (GOT) are the commonly measured transaminases. Changes in serum and tissue transaminase activity are supposed to reflect the status of tissue or the organ. Excessive destruction of the tissue leads to increase in the activity of these enzymes in serum. Scanning of the literature reveals that transaminase activity is measured in different types of target tissue, in liver as secondary tissue and in serum. Experimental methods used for the study are also different. Gupta and coworkers (329) have measured transaminase activity in serum obtained from formaldehyde-injected rats. Elevated transaminase activity and its reversal by standard reference drugs and test drugs has been reported by these authors. However, this decrease was reported not to correlate with the anti-inflammatory effect of the drugs. Transaminase activity in oedema tissue obtained from carrageenin-injected paw and granulation tissue obtained from cotton pellet-implanted rats which were target tissue for inflammation, and in liver (as secondary tissue) have been measured by Naik and Sheth (327). They have reported that transaminase activity increases significantly after the induction of inflammation and this elevation is significantly reversed after the administration of test drugs and reference standards. According to them elevation in GOT activity and its reversal by the test drugs may be relevant to anti-inflammatory activity since the drug treatment decreased activity of only GPT in normal rats while in cotton pellet-implanted rats the decrease was observed in both GOT and GPT activity. Nataraja (211) could not find correlation between changes in transaminase activity and anti-inflammatory effect. In the present study, transaminase activity has been measured in granulation tissue obtained from PVC cup-implanted rats. GOT activity was not altered by both test extracts and reference standards while dexamethasone(DEX) increased GPT activity. No such effect could be observed with phenylbutazone (PBZ). According to a study by Savitskii (331), GOT and GPT activity increased in mitochondrial fraction of liver tissue homogenate and decrease was observed in sol fraction. These reports make it clear that there is no uniformity between the application of inflammatory stimulus and changes observed in transaminase activity and their modification by anti-inflammatory drugs.
It has been reported that transaminases influence the continuous formation of biologically active polypeptides and kinin-like substances which are well known mediators of inflammation (327,332). They have also been assigned important role in the formation of mucopolysaccharides (329) which are important constituents of connective tissues. Diminished formation of kinin-like substances may lead to attenuation of acute inflammatory response. Inhibition of mucopolysaccharide biosynthesis will lead to reduced formation of connective tissue causing impairment in the repair phase of inflammation and ultimately leading to anti-inflammatory activity.

Measurement of transaminase activity was carried out in serum and liver of cotton pellet implanted (CP) and carrageenin induced granuloma pouch bearing rats (GP). In addition the transaminase activity was also measured in the granulation tissue obtained from GP rats.

**GPT activity:**

Increase in GPT activity in serum was observed in CP rats. In contrast to this, no change could be observed in GP rats. This again shows that change in enzyme activity may depend on the nature of inflammatory stimulus. This elevation in GPT activity was inhibited by ME of VAL and PBZ treatment. ME of VNDL did not affect serum GPT activity while betamethasone (BET) increased serum GPT activity. This results support the observation of earlier workers that changes in GPT activity do not correlate well with anti-inflammatory activity.

GPT activity significantly increased in liver homogenate from CP group in comparison to normal control. This finding is in conformity with the report of Naik and Sheth (327). ME of VAL produced significant antagonism of this elevation in GPT activity. The antagonism observed with ME of VNDL was statistically non-significant. But the GPT activity in GP rats was lower in comparison to the normal control. In extract treated group, GPT activity was increased but not in reference standard administered groups. This difference between the effect of reference standard drugs and test extracts suggests that this activity may not be related to the anti-inflammatory activity of the extracts.

ME of VAL did not affect GPT activity in granulation tissue. A decrease at lower dose level and no change at higher dose was observed with ME of VNDL. The enzyme activity was significantly decreased in indomethacin (IND) and BET treated groups. But the decrease noted with PBZ was not statistically significant. Effect on GPT activity in the target tissue did not correlate with the anti-inflammatory activity observed.
GOT activity:

Statistically non-significant decrease in GOT activity in serum was observed in CP rats and no change could be observed in GP rats in comparison to normal rats. The extracts and PBZ did not influence serum GOT activity significantly in CP rats. In contrast to this, increase in serum GOT activity was observed in BET administered rats. In GP rats, lower dose of ME of VAL and higher dose of ME of VNDL decreased serum GOT activity. Indomethacin and PBZ had no influence but BET decreased serum GOT activity.

The different response observed between the drugs within the model and difference in the response observed between the models makes it difficult to relate these observations with the anti-inflammatory activity.

In contrast to the above observation, homogenous data were obtained with regards to GOT activity in the liver homogenate of CP rats. In this model, significant increase in liver GOT activity was observed in CP rats in comparison to normal rats. This increase was significantly antagonised by both test extracts and reference standard drugs. However, liver GPT activity was not altered in GP rats in comparison to normal rats and in this model the extract and reference standard did not modify GOT activity significantly.

GOT activity in granulation tissue significantly decreased in ME of VNDL and reference standard administered rats. The decrease observed with ME of VAL was not statistically significant.

Analysis of the results failed to reveal any correlation between transaminase activity as a whole and the anti-inflammatory activity observed with the test drugs. Wide variation observed in the results points out the necessity of conducting detailed studies with a view to ascertain whether changes observed in transaminase activity have any relationship with the anti-inflammatory activity or not. This may require employing uniform measuring technique under standardised experimental conditions with suitable models of experimental inflammation.

Acid phosphatase activity:

Lysosomal enzymes released by inflammatory cells at the site of inflammation play important role in the development of both acute and chronic inflammation. Hydrolytic enzymes released as a result of death of the cell or after interaction with noxious stimulus in a selective manner may be responsible for the tissue damage, degradation and remodelling observed during chronic inflammation. Increased lysosomal enzyme activity has been reported in rheumatoid synovia and synovial membrane and other inflamed tissues (327, 333). Similar increase has been reported in certain types of experimental inflammation (327, 334, 335). Stabilization of lysosomal membrane resulting in decrease in the release of lysosomal content and inhibition of
the factors released from lysosome has been proposed as one of the mechanisms through which NSAIDs produce anti-inflammatory effect (327, 336). Acid phosphatase (ACPase) is one of the commonly employed parameters as a biochemical marker to assess the damage to the lysosomal membrane (327, 334). It is reported to be localised almost exclusively in lysosomal particles and its release is parallel to the release of other lysosomal hydrolases (327, 337).

According to the report of Naik and Sheth (327), ACPase activity increased significantly in oedema tissue obtained from carrageenin-injected paw and granulation tissue from cotton pellet-implanted rats which were used as target tissues and as well as in liver homogenate which was used as a secondary tissue. This elevated ACPase activity was reported to be antagonised by test drugs and reference standards.

In the present study, ACPase activity was measured in serum and liver homogenates obtained from CP and GP rats in granulation tissue obtained from GP rats. Acid phosphatase activity increased significantly in serum of CP rats in comparison to normal rats. The increase in serum ACPase observed in GP rats was not statistically significant. The increase observed in CP rats was significantly antagonised by treatment with the extracts while reference standards did not affect the enzyme activity significantly. In GP rats, ME of VNDL produced marked decrease in serum ACPase. The decrease observed with ME of VAL was not statistically significant. Decrease in enzyme activity was also observed in PBZ and BET treated groups while IND did not produce significant effect. ACPase activity in liver homogenate from CP rats was lower in comparison to normal rats. This observation is not in conformity with the earlier report (327). The reason for the difference is not clear. The results obtained with test extracts and reference standards were divergent. ME of VAL further lowered liver ACPase activity. While ME of VNDL significantly increased it, PBZ had no effect and BET increased the enzyme activity. In GP rats, ACPase activity did not increase in comparison to normal rats. The extracts did not alter the enzyme activity. PBZ and IND decreased it and BET did not produce significant inhibition. ACPase activity in the granulation tissue, which was used as the target tissue, increased in higher dose ME of VAL and IND administered groups. No significant change could be observed in other groups. Wide variations in the activity observed with test extracts and reference standards makes it very difficult to draw any meaningful conclusion. The variation noted in the GP rats may be due to different nature of the inflammatory stimuli. However, it is difficult to explain the difference in the nature of ACPase activity observed in liver homogenate in the present study and previous report.

Effect on serum orosomucoid level and ceruloplasmin activity:

Inflammatory arthropathies are traditionally treated with NSAIDs as first line of treatment (227). Though they offer symptomatic relief they do not significantly influence the disease progression in rheumatoid arthritis. This has lead to undertaking of number of studies to find
out drugs with specific anti-rheumatic activity (227, 232). Drugs that are capable of arresting the disease progression came to be identified as disease modifying anti-rheumatic drugs (227, 232). The disease modifying drugs are supposed to lower RF titers, acute phase proteins and activated circulating lymphocytes (338). Measurement of acute phase proteins in Freund's adjuvant induced arthritis and other chronic inflammatory models has been used as a testing procedure for evaluating disease modifying anti-rheumatic drugs (338, 339). Recently, clobuzarit an experimental drug has been reported to possess such an effect (338). This drug lowered elevated acute phase protein levels in three experimental models of chronic inflammation. Acute phase proteins or acute phase reactants are a group of plasma proteins whose level consistently rises as a physiological response to different types of inflammatory states (340). This group consists of C-reactive protein, anti-chymotrypsin, a-acid glycoprotein (orosomucoid), fibrinogen, haptoglobin, a-acid antitrypsin ceruloplasmin and third complement factor. Some of the constituents of this group act as antiproteases to regulate the tissue destructive potential of lysosomal enzymes released from phagocytic inflammatory cells. These anti-proteases are present in serum and in synovial fluid and inhibit protease activity by binding to them and covering their active sites (197). Acute phase response seems to involve a series of complex responses including a direct effect of interleukin1 (IL1) on the liver. IL1 stimulates hepatocytes to produce acute phase protein IL6, tumour necrosis factor and glucocorticoids. Increased production of some of these factors enhance activation and migration of mononuclear cells to the site of inflammation. Activated mononuclear cells may release increased amount of monokines leading to amplification of the response (338). Disease modifying drugs may act by inhibiting the activation of mononuclear cells (338). Ability of the test drugs to lower ESR and elevated levels of acute phase proteins has been used as a biochemical marker to assess their efficacy in rheumatoid arthritis (RA) (338, 339). In the present study, effect of test extracts was noted on serum orosomucoid level and ceruloplasmin activity two representative acute phase proteins in cotton pellet implanted (CP) and carrageenin-induced granuloma pouch bearing (GP) rats to assess them for possible disease modifying anti-rheumatic effect. Marked elevation in serum orosomucoid level was observed in both CP and GP rats in comparison to normal rats. In CP rats, decrease in orosomucoid level was observed in both the extracts administered groups but the decrease did not reach statistically significant level. In GP rats also decrease was observed with both the extracts but it was statistically significant only in lower dose (100 mg kg\(^{-1}\)) ME of VAL administered group. Reference standard drugs did not modify serum orosomucoid level in CP rats. In GP rats, statistically non-significant decrease was observed in BET administered group while marginal increase was observed in IND and BET administered groups. Significant increase in serum ceruloplasmin activity was also observed. The decrease in ceruloplasmin activity observed in extract administered groups did not attain statistical significance. The results point towards the possibility of presence of disease modifying effect in ME of VAL. However ME of VAL did not affect Freund's adjuvant-induced arthritis though it was effective in suppressing granulation tissue formation in CP and GP rats and inhibiting SRBC induced immunological oedema. Hence, it would not be prudent to ascribe disease-modifying effect
to the extract. Further studies involving measurement of all the acute phase proteins, albumin, prealbumin and iron levels and ESR in different models of chronic inflammation would help in assessing the possible disease modifying effect in the extracts.

**Effect on connective tissue constituents:**

Formation of connective tissue is one of the main features of repair phase of inflammation. This involves formation and maturation of granulation tissue. Both cellular and intercellular substance are present in connective tissue. Fibroblasts and mast cells are the main cell types and collagen, elastin, mucopolysaccharides and electrolytes are the main inter cellular substances present (341, 204). Collagen are the fibrillar component of connective tissue in the joints (204). Collagen molecules are semirigid, rod-shaped molecules consisting of three polypeptide chains arranged in characteristic and unique helical configuration. Collagen molecule is formed through a series of complex reactions involving formation of precursor forms termed procollagens (341, 342). The process involves incorporation of aminoacids proline, glycine and lyine into the primary chain and a unique post transitional modification of component aminoacids. The most important of these is introduction of hydroxyl group in the 4th position of specific polyl residues. 4-hydroxyproline residues are considered important for the stabilization of collagen helix. Specific lysine residues are also modified by hydroxylation in the 5 position to form hydroxylysine. Glycolisation of some of the hydroxylysine molecules also occurs. Lysine, hydroxylysine and their derivatives are involved in inter and intramolecular cross linking. The formed intermediates are translocated to the site of assembly to form triple helix and finally conversion of procollagen to collagen (342).

Mucopolysaccharides (high molecular weight carbohydrates) are the other major components of connective tissue. They form the major part of ground substance of the inter fibrillar matrix and are composed of glycosaminoglycan portion linked to a core protein. This core protein is called proteoglycan subunit. The glycosaminoglycans are polysaccharides containing hexosamine and hexuronic acids (204, 341). The mucopolysaccharides are formed through the following steps: 1) synthesis of the intermediates for glycosaminoglycans, ii) formation of protein polysaccharide linkage region iii) elongation of chains by sequential addition of the hexosamine and uronic acid residues and iv) sulfation of the glycosaminoglycans (342).

In RA, derangement of connective tissue metabolism is one of the main features (204). Changes may occur in the proportion of type I and type II collagen in the joints. Cartilage of normal joints produces predominantly type II collagen. In contrast to this, synovial membrane in arthritic joints may produce large amounts of type I collagen, which is more susceptible to synovial collagenase. Its increased presence in the diseased cartilage may be one of the causes of enhanced tissue degradation observed in RA (341).
Measurement of hydroxyproline and hexosamine content of the granulation tissue is normally undertaken to assess the test drugs effect on collagen and mucopolysaccharide metabolism respectively in inflammatory conditions. Increased level of hydroxyproline has been reported in different types of inflammatory conditions (325, 334). Anti-rheumatic drugs are reported to modulate the biosynthesis of connective tissue components. In the present study, effect of test extracts on hydroxyproline content as an index of collagen metabolism and hexosamine content as an index of mucopolysaccharides metabolism in granulation tissue obtained from GP rats was assessed.

The data obtained from the study were presented in two forms, the first being expression of data as absolute content of hydroxyproline and hexosamine in granulation tissue and the second being expression of data as relative content of hydroxyproline and hexosamine (i.e. ug/g tissue) in granulation tissue. Absolute content is supposed to reflect the changes in the components of connective tissue. Relative values are supposed to provide information about the nature of test drug-induced modulation on the biosynthesis of collagen and mucopolysaccharides. The data obtained during the study show that both the extracts produced significant decrease in hydroxyproline content when the data were presented as absolute values. However, when the data were presented as relative values significant decrease could be observed only in higher dose ME of VNDL treated groups. This may suggest that ME of VNDL decreased collagen content in connective tissue through inhibition of biosynthesis of hydroxyproline. While decrease observed in ME of VAL may be due to modulation of organisation differentiation and intracellular translocation of collagen. It is also possible that the decrease may be due to inhibition of proliferation of fibroblasts (343).

Decrease in absolute hexosamine content of granulation tissue was observed in ME of VAL treated group. However, the decrease was not dose dependent. The decrease observed with lower dose was statistically significant. The decrease observed with higher dose did not reach the level of statistical significance. Absolute hexosamine content was not affected in ME of VNDL and reference standard administered groups. None of the drugs could lower hexosamine content in granulation tissue when the data were expressed as relative values. On the contrary, increase was observed with IND and BET. The results indicate that the test drugs did not possess modulatory effect on biosynthesis of hexosamine. The decrease in absolute hexosamine content observed in ME of VAL may represent modulation of mucopolysaccharide synthesis at the site of assembly of intermediates. The increase in relative content on hexosamine observed with reference standard may be explained as a consequence of drugs inhibitory effect on cell emigration and proliferation causing decrease in the weight of granuloma without affecting biosynthesis of hydroxyproline and hexosamine.
Effect of RNA and DNA:

Nucleic acid content of a given tissue is considered to represent cellular content of it. Drugs causing decrease in cellular content through either cytotoxic effect or by inhibiting their migration are reported decrease DNA content of that tissue (326). Hence, RNA and DNA content of the granulation tissue obtained from GP rats was estimated. Significant decrease in RNA and DNA content of granulation tissue when expressed as absolute values was observed in both test extract and reference standards administered groups. However, when the data were presented as relative value, only the decrease in DNA content observed in lower dose ME of VNDL and PBZ-treated groups was significant. Decrease in relative RNA content was observed in ME of VNDL, BET and IND administered groups. The results indicate that the test drug treatment caused significant decrease in the cellular content of granulation tissue in GP rats.

Evaluation of ME of both Vitex altissima and Vitex negundo leaf revealed that both possess significant anti-inflammatory activity. They have modulatory effect on all the three phases of inflammation, i.e. they suppress fluid exudation, cell emigration and cell proliferation and connective tissue formation. Their mechanism of action appears to be more or less similar, not withstanding quantitative and qualitative difference in certain aspects.