Results and Observations
RESULTS AND OBSERVATIONS

1. PHARMACOGNOSTICAL STUDY OF POWDERED DRUGS:

The powdered microscopy of Amalaki (*Emblica officinalis* Gaertn.), Bibhitaka (*Terminalia belerica* (Gaertn) Roxb.), Haritaki (*Terminalia chebula* Retz.), Guduchi (*Tinospora cordifolia* (Willd.) Miers.), Neem (*Azadirachta indica* A. Juss.) and Patola (*Trichosanthes dioica* Roxb.) were carried for authenticity of plant materials and the observed powder characters were drawn by using camera lucida.

1.1. Amalaki (*Emblica officinalis* Gaertn.)

Powder of the pericarp of fruits showed characters like 4 to 6 angled moderately thick-walled cells of epicarp in surface view each loaded with prismatic crystal of silica; round to oval parenchyma cells of the mesocarp filled with tannin and few prisms; circular to cylindrical pitted parenchyma; group of stone cells with broad lumen; fragments of scalariform vessels attached with thin-walled fibers; fragments or entire narrow lumened fibers with or without slanted pits and sharp pointed ends; highly lignified columnar to cylindrical long pillar like pitted sclerieds of endocarp and few silica crystals scattered as such throughout powder.

1.2. Bibhitaka (*Terminalia belerica* (Gaertn) Roxb.)

Powder of the pericarp of fruits showed characters like abundant long and short, short and slightly bent bulbous base trichome; thick-walled 4-angled to circular epicarp cells some containing rosette crystals of calcium oxalate; thin-walled rounded parenchyma of mesocarp few cells filled with tannin and some loaded with prismatic crystals of calcium oxalate; elliptical scleriedal fibers; sclerieds and stone cells of various sizes and shapes with circular pits on the walls; pitted parenchyma; fragments of simple pitted and spiral vessels and rosette crystals of calcium oxalate scattered throughout powder.

1.3. Haritaki (*Terminalia chebula* Retz.)

Powder of the pericarp of fruits showed characters like penta to hexagonal thick-walled cells of epicarp in surface view; round, oval to elliptical simple parenchyma from mesocarp loaded with simple to 3-compound starch grains; few
Results and Observations

round to branched pitted parenchyma; group of pitted sclerieds; fragments of highly pitted scleriedal fibers; few narrow elongated scleriedal fibers; thin-walled and non-pitted fragments of criss-cross fibers and abundant simple round to oval and 2 to 4 compound starch grains scattered as such in the powder.

1.4. **Guduchi (Tinospora cordifolia (Willd.) Miers.)**

Powder of the mature stem showed characters like polygonal several strata of cork cells placed one above the other in surface view; few cork cells in surface view loaded with 1 to 3 prismatic crystals of calcium oxalate; rectangular cells of cork in sectional view; moderately thick-walled round to oval cortical parenchyma loaded with simple to compound starch grains; cells of the cortical parenchyma attached with comparatively large sized mucilage cell; tiny stone highly pitted stone cells with broad lumen and elongated highly pitted sclereids with narrow lumen; long sharp ended fibers with transverse slit-like pits on their wall; entire or fragments of large reticulately thickened vessels and abundant simple oval rounded to elliptical, 2 to 3 compound and aggregate starch grains, few with triangular pits, are scattered throughout the powder.

1.5. **Nimba (Azadirachta indica A. Juss.)**

Powder of the bark showed polygonal cork cells arranged one above the other in surface view and few fragments in transversely cut mode showed tabular cells; rounded parenchyma cells of the cortex few containing simple to 3-compound starch grains and some filled with tannin; septate phloem fibres with very thick wall and narrow lumen filled with reddish brown contents; transversely cut medullary ray cells showed usually multiseriate cells; transversely striated stone ells and sclereids of various sizes and shapes with prominently or sometimes lightly pitted wall; some stone cells included with prismatic crystal of calcium oxalate within the lumen; crystal fibres- fibres attached with parenchyma loaded with prismatic crystals of calcium oxalate; fragments or entire pitted and spiral vessels; tannin bodies scattered throughout the powder; abundant prismatic crystals of calcium oxalate and simple to 3-compound starch grains scattered throughout the powder.
Fig. 1. Powder microscopy of *Emblica officinalis* fruits. a, epidermis in surface view; b, mesocarp parenchyma; c, starch grains; d, pitted parenchyma; e, stone cells; f, prismatic crystals of silica; g, sclereids; h, pitted vessels attached to parenchyma; i, cell from the phloem; j, fibres with and without

Fig. 2. Powder microscopy of *Terminalia belerica* fruits. a, phloem parenchyma with rosette crystals of calcium oxalate; b, different types stone cells and sclereids; c, rosette crystals of calcium oxalate; d, starch grains; e, tannin body; f, group of pitted and spiral vessels; g, bulbous base trichomes; h, mesocarp parenchyma with starch grains and prismatic crystals of calcium oxalate; i, sclereids with narrow lumen; j, fragment of pitted vessels.
Fig. 3. Powder microscopy of *Terminalia chebula* fruit. 

**a,** epicarp in surface view; 
**b,** group of porous sclerieds; 
**c,** tannin body; 
**d,** mesocarp parenchyma with starch grains and tannin; 
**e,** pitted parenchyma; 
**f,** pitted sclerieds; 
**g,** criss-cross fibres; 
**h,** starch grains; 
**i,** fibre;

Fig. 4. Powder microscopy of *Tinospora cordifolia* stem. 

**a,** long pitted fibre; 
**b,** cork in sectional view; 
**c,** phloem parenchyma; 
**d,** cortical parenchyma filled with starch grains; 
**e,** starch grains; 
**f,** fragment of pitted scleried; 
**g,** pitted parenchyma cell; 
**h,** cork in surface view; 
**i,** parenchyma attached to mucilage cell; 
**j,** cork in surface view showing prismatic crystals of calcium oxalate; 
**k,** different types of sclerieds; 
**l,** prismatic crystals of calcium oxalate; 
**m,** entire pitted vessel.
Fig. 5. Powder microscopy of *Azadiracta indica*. a, long thick-walled septate fibre; b, prismatic crystals of calcium oxalate; c, tannin cells; d, crystal fibre; e, cortical parenchyma with starch grains; f, fragment of pitted sclerieds; g, phloem parenchyma; h, fragment of pitted vessel; i, transversely cut cork; j, stone cells without pits; k, cork in surface view; l, group of thin-walled scleried; m, group of thick-walled sclerieds; n, starch grains.

Fig. 6. Powder microscopy of *Trichosanthes dioica* leaf. a, transversely cut lamina showing palisade and mesophyll loaded with prismatic crystals of calcium oxalate; b, thick-walled fibre; c, group of pitted sclerieds; d, fragment of resin canal; e, parenchyma with colouring matter; f, pitted parenchyma; g, fragment of upper epidermis showing stomata; h, glandular trichome with multicellular head; i, prismatic crystals of calcium oxalate; j, different types of glandular trichomes; k, a papillae; l, epidermis showing papilla and cicatrix; m, multicellular covering trichomes.
1.6. **Patola (Trichosanthes dioica Roxb.)**

Powder showed characters like fragments of straight-walled to slightly sinous walled epidermis with anomocytic stomata and few papillae. Plenty of multicellular and uniseriate covering trichomes and multicellular glandular trichomes with multicellular base and unicellular head, some with unicellular base and celled head; transversely cut lamina showed single layer of palisade and mesophyll below it embedded with prismatic crystal of calcium oxalate, fragments of septate resin canals, fragments of spiral vessels and conical parenchyma with micro sphenoidal crystals of calcium oxalate and mesophyll cell contains brown coloured content.

2. **PHYTOCHEMICAL STUDIES:**

Triphala equal, Triphala unequal powder (80 mesh fine powder) and Chinnodbhavadi kwath powder (coarse powder) were used for estimation of various physicochemical parameters, phytochemical qualitative organic analysis, heavy metal analysis and HPTLC study.

**Table-1**

Physicochemical parameters of Triphala equal, Triphala unequal and Chinnodbhavadi kwath churna.

<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>Parameters (% w/w)</th>
<th>Triphala equal</th>
<th>Triphala unequal</th>
<th>Chinnod. kwath</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Loss on drying</td>
<td>5.5 %</td>
<td>5.20 %</td>
<td>10.1 %</td>
</tr>
<tr>
<td>2.</td>
<td>Total ash value</td>
<td>6.1 %</td>
<td>5.81 %</td>
<td>18.2 %</td>
</tr>
<tr>
<td>3.</td>
<td>Acid insoluble ash</td>
<td>0.18 %</td>
<td>0.175 %</td>
<td>1.10 %</td>
</tr>
<tr>
<td>4.</td>
<td>Water soluble extractive</td>
<td>45.20 %</td>
<td>49.71 %</td>
<td>51.30 %</td>
</tr>
<tr>
<td>5.</td>
<td>Alcohol soluble extractive</td>
<td>39.78 %</td>
<td>38.35 %</td>
<td>35.45 %</td>
</tr>
</tbody>
</table>

Physicochemical parameters in all three Triphala formulations showed values almost in similar fashion except kwath showed higher value of total ash content compared to other two formulations (table-1).
Results and Observations

Table-2

Preliminary phytochemical qualitative test for presence of phyto-constituents in formulations

<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>Test</th>
<th>Triphala equal</th>
<th>Triphala unequal</th>
<th>Chinnod. kwath</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Tannins</td>
<td>+ ve</td>
<td>+ ve</td>
<td>+ ve</td>
</tr>
<tr>
<td>2.</td>
<td>Flavanoids</td>
<td>+ ve</td>
<td>+ ve</td>
<td>+ ve</td>
</tr>
<tr>
<td>3.</td>
<td>Saponins</td>
<td>+ ve</td>
<td>+ ve</td>
<td>+ ve</td>
</tr>
<tr>
<td>4.</td>
<td>Terpenoids</td>
<td>+ ve</td>
<td>+ ve</td>
<td>+ ve</td>
</tr>
<tr>
<td>5.</td>
<td>Sterols</td>
<td>+ ve</td>
<td>+ ve</td>
<td>+ ve</td>
</tr>
<tr>
<td>6.</td>
<td>Alkaloids</td>
<td>- ve</td>
<td>- ve</td>
<td>+ ve</td>
</tr>
<tr>
<td>7.</td>
<td>Glycosides/Sugar</td>
<td>+ ve</td>
<td>+ ve</td>
<td>+ ve</td>
</tr>
<tr>
<td>8.</td>
<td>Carboxylic acid</td>
<td>+ ve</td>
<td>+ ve</td>
<td>+ ve</td>
</tr>
</tbody>
</table>

Sign (+ve) means present and (−ve) means absent

Table-3

Analysis of trace heavy metal constituents in the Triphala formulations.

<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>Heavy metals (PPM)</th>
<th>Triphala equal</th>
<th>Triphala unequal</th>
<th>Kwath</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Lead</td>
<td>0.589</td>
<td>0.628</td>
<td>0.842</td>
</tr>
<tr>
<td>2.</td>
<td>Cadmium</td>
<td>0.0172</td>
<td>0.0145</td>
<td>0.021</td>
</tr>
<tr>
<td>3.</td>
<td>Arsenic</td>
<td>0.195</td>
<td>0.215</td>
<td>0.362</td>
</tr>
<tr>
<td>4.</td>
<td>Mercury</td>
<td>0.078</td>
<td>0.065</td>
<td>0.071</td>
</tr>
</tbody>
</table>

The concentration of trace heavy metals such as lead (Pb), cadmium (Cd), arsenic (As) and mercury (Hg) present in formulations were analyzed by Atomic Absorption Spectrophotometer. The results were expressed in PPM level (table-3).
HPTLC standardization was carried out by using methanolic extract of all six ingredients and three Triphala formulations. The plate was developed in Toluene: Ethyl acetate: Formic acid (5:5:1) solvent system. The Rf value observed at 366 nm and at visual wave length were shown in table-4. Gallic acid used as reference standard and was observed at Rf 0.478 at 366 nm and 0.486 at visual wavelength. Ingredients of Triphala and Triphala formulations also have shown the same Rf as observed in gallic acid.

### Table-4

Observation of TLC profile of individual ingredients, gallic acid and three Triphala formulations at 366 nm and after post chromatographic derivatization with vanillin-sulphuric acid

<table>
<thead>
<tr>
<th>Track</th>
<th>At 366 nm</th>
<th>After derivatization with vanillin-sulphuric acid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of</td>
<td>Rf values</td>
</tr>
<tr>
<td></td>
<td>spot</td>
<td></td>
</tr>
<tr>
<td>I- Emblica officinalis</td>
<td>4</td>
<td>0.108 (f), 0.348 (b), 0.478 (db), 0.630 (b)</td>
</tr>
<tr>
<td>II- Terminalia belerica</td>
<td>6</td>
<td>0.174 (f), 0.348 (b), 0.478 (db), 0.565 (pb), 0.630 (b), 0.695 (pb)</td>
</tr>
<tr>
<td>III- Terminalia Chebula</td>
<td>6</td>
<td>0.087 (b), 0.152 (pb), 0.348 (b), 0.369 (f), 0.478 (db), 0.630 (b)</td>
</tr>
<tr>
<td>IV- Tinospora codifolia</td>
<td>3</td>
<td>0.108 (w), 0.630 (b), 0.93 (pb)</td>
</tr>
<tr>
<td>V- Azadirachta indica</td>
<td>3</td>
<td>0.108 (pb), 0.630 (b), 0.93 (pb)</td>
</tr>
<tr>
<td>VI- Trichosanthes dioica</td>
<td>4</td>
<td>0.108 (pb), 0.217 (pb), 0.630 (b), 0.956 (p)</td>
</tr>
<tr>
<td>VII- Triphala equal</td>
<td>7</td>
<td>0.108 (b), 0.174 (f), 0.348 (b), 0.478 (db), 0.565 (pb), 0.630 (b), 0.695 (pb)</td>
</tr>
<tr>
<td>VIII- Triphala unequal</td>
<td>5</td>
<td>0.108 (b), 0.478 (db), 0.565 (pb), 0.630 (b), 0.695 (pb)</td>
</tr>
<tr>
<td>IX- Chinnod kwath</td>
<td>9</td>
<td>0.108 (b), 0.174 (f), 0.348 (b), 0.478 (db), 0.565 (pb), 0.630 (b), 0.695 (pb), 0.913 (pb), 0.956 (p)</td>
</tr>
<tr>
<td>X- Gallic acid</td>
<td>1</td>
<td>0.478</td>
</tr>
</tbody>
</table>

f- Florescent blue, b- Blue, db- Dark blue, pb- Pale blue, p- Pink, g- Gray, br- Brown, pbr- Pale brown, v- Violet
HPTLC fluorescence image under the excitation wavelength 254 nm

HPTLC image after post chromatographic derivatization with vanillin sulphuric acid

HPTLC image after post chromatographic derivatization with vanillin sulphuric acid
Track 1, Analysis a: Track 1

<table>
<thead>
<tr>
<th>Peak</th>
<th>Start Rf</th>
<th>H</th>
<th>Max Rf</th>
<th>H</th>
<th>[%]</th>
<th>Start Rf</th>
<th>H</th>
<th>End Rf</th>
<th>H</th>
<th>F</th>
<th>Area [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.01</td>
<td>0</td>
<td>0.08</td>
<td>228.9</td>
<td>12.31</td>
<td>0.11</td>
<td>0.5</td>
<td>8263.6</td>
<td>11.88</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.15</td>
<td>15.7</td>
<td>0.19</td>
<td>165.8</td>
<td>8.92</td>
<td>0.20</td>
<td>161.2</td>
<td>3703.2</td>
<td>5.33</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.20</td>
<td>162.5</td>
<td>0.20</td>
<td>162.6</td>
<td>8.75</td>
<td>0.23</td>
<td>113.6</td>
<td>3147.0</td>
<td>4.53</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.23</td>
<td>113.6</td>
<td>0.26</td>
<td>168.9</td>
<td>9.09</td>
<td>0.29</td>
<td>106.2</td>
<td>5693.7</td>
<td>8.19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.29</td>
<td>106.2</td>
<td>0.35</td>
<td>347.8</td>
<td>18.71</td>
<td>0.43</td>
<td>0.1</td>
<td>20567.1</td>
<td>29.60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.43</td>
<td>1.1</td>
<td>0.49</td>
<td>551.4</td>
<td>29.67</td>
<td>0.53</td>
<td>40.3</td>
<td>20970.4</td>
<td>30.15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>0.61</td>
<td>4.8</td>
<td>0.63</td>
<td>59.0</td>
<td>3.18</td>
<td>0.66</td>
<td>5.8</td>
<td>1219.5</td>
<td>1.75</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>0.79</td>
<td>0.6</td>
<td>0.83</td>
<td>113.4</td>
<td>6.10</td>
<td>0.87</td>
<td>3.3</td>
<td>3355.0</td>
<td>4.82</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>0.90</td>
<td>8.6</td>
<td>0.97</td>
<td>60.7</td>
<td>3.27</td>
<td>0.99</td>
<td>24.1</td>
<td>2603.9</td>
<td>3.74</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Total height = 1858.5
Total area = 69543.4

Track 2, Analysis b: Track 2

<table>
<thead>
<tr>
<th>Peak</th>
<th>Start Rf</th>
<th>H</th>
<th>Max Rf</th>
<th>H</th>
<th>[%]</th>
<th>Start Rf</th>
<th>H</th>
<th>End Rf</th>
<th>H</th>
<th>F</th>
<th>Area [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.02</td>
<td>0.5</td>
<td>0.04</td>
<td>85.6</td>
<td>7.49</td>
<td>0.05</td>
<td>54.8</td>
<td>1305.0</td>
<td>3.14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.05</td>
<td>54.8</td>
<td>0.06</td>
<td>69.1</td>
<td>6.05</td>
<td>0.07</td>
<td>0.7</td>
<td>756.4</td>
<td>1.82</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.09</td>
<td>9.5</td>
<td>0.14</td>
<td>68.3</td>
<td>5.99</td>
<td>0.18</td>
<td>22.5</td>
<td>2746.5</td>
<td>6.61</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.20</td>
<td>15.3</td>
<td>0.22</td>
<td>34.8</td>
<td>3.04</td>
<td>0.24</td>
<td>8.8</td>
<td>798.0</td>
<td>1.90</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.27</td>
<td>22.3</td>
<td>0.35</td>
<td>256.5</td>
<td>22.47</td>
<td>0.43</td>
<td>0.0</td>
<td>17659.0</td>
<td>42.52</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.44</td>
<td>23.1</td>
<td>0.48</td>
<td>377.9</td>
<td>33.12</td>
<td>0.53</td>
<td>23.2</td>
<td>12171.9</td>
<td>29.31</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>0.53</td>
<td>23.1</td>
<td>0.56</td>
<td>47.1</td>
<td>4.13</td>
<td>0.59</td>
<td>0.6</td>
<td>1417.3</td>
<td>3.41</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>0.60</td>
<td>4.4</td>
<td>0.63</td>
<td>59.1</td>
<td>5.17</td>
<td>0.65</td>
<td>0.4</td>
<td>1106.4</td>
<td>2.66</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>0.68</td>
<td>3.7</td>
<td>0.71</td>
<td>98.0</td>
<td>8.59</td>
<td>0.75</td>
<td>0.6</td>
<td>2100.9</td>
<td>5.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0.93</td>
<td>14.0</td>
<td>0.97</td>
<td>45.0</td>
<td>3.94</td>
<td>1.00</td>
<td>0.8</td>
<td>1476.2</td>
<td>3.55</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Total height = 1141.3
Total area = 41527.5
### Track 3, Analysis c: Track 3

<table>
<thead>
<tr>
<th>Peak</th>
<th>RF</th>
<th>H</th>
<th>RF</th>
<th>H</th>
<th>%</th>
<th>RF</th>
<th>H</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.02</td>
<td>0.7</td>
<td>0.04</td>
<td>100.6</td>
<td>6.68</td>
<td>0.05</td>
<td>70.9</td>
<td>1656.2</td>
</tr>
<tr>
<td>2</td>
<td>0.05</td>
<td>70.9</td>
<td>0.07</td>
<td>120.5</td>
<td>8.01</td>
<td>0.08</td>
<td>8.8</td>
<td>1464.1</td>
</tr>
<tr>
<td>3</td>
<td>0.08</td>
<td>1.6</td>
<td>0.10</td>
<td>35.0</td>
<td>2.33</td>
<td>0.11</td>
<td>32.1</td>
<td>456.1</td>
</tr>
<tr>
<td>4</td>
<td>0.11</td>
<td>32.1</td>
<td>0.15</td>
<td>189.4</td>
<td>32.59</td>
<td>0.17</td>
<td>90.1</td>
<td>5565.6</td>
</tr>
<tr>
<td>5</td>
<td>0.17</td>
<td>90.1</td>
<td>0.18</td>
<td>103.1</td>
<td>6.85</td>
<td>0.21</td>
<td>0.0</td>
<td>1727.9</td>
</tr>
<tr>
<td>6</td>
<td>0.24</td>
<td>7.5</td>
<td>0.37</td>
<td>248.0</td>
<td>16.48</td>
<td>0.42</td>
<td>46.0</td>
<td>16775.1</td>
</tr>
<tr>
<td>7</td>
<td>0.42</td>
<td>46.0</td>
<td>0.48</td>
<td>469.4</td>
<td>31.19</td>
<td>0.51</td>
<td>66.9</td>
<td>17304.6</td>
</tr>
<tr>
<td>8</td>
<td>0.51</td>
<td>66.9</td>
<td>0.53</td>
<td>98.4</td>
<td>6.34</td>
<td>0.58</td>
<td>0.2</td>
<td>2598.0</td>
</tr>
<tr>
<td>9</td>
<td>0.58</td>
<td>0.1</td>
<td>0.62</td>
<td>93.8</td>
<td>6.23</td>
<td>0.65</td>
<td>2.3</td>
<td>2170.7</td>
</tr>
<tr>
<td>10</td>
<td>0.93</td>
<td>12.3</td>
<td>0.97</td>
<td>46.9</td>
<td>3.11</td>
<td>1.00</td>
<td>8.3</td>
<td>1634.5</td>
</tr>
</tbody>
</table>

Total height = 1505.1 total area = 51292.8

### Track 4, Analysis d: Track 4

<table>
<thead>
<tr>
<th>Peak</th>
<th>RF</th>
<th>H</th>
<th>RF</th>
<th>H</th>
<th>%</th>
<th>RF</th>
<th>H</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.01</td>
<td>179.0</td>
<td>0.01</td>
<td>190.4</td>
<td>40.29</td>
<td>0.02</td>
<td>0.2</td>
<td>1056.9</td>
</tr>
<tr>
<td>2</td>
<td>0.07</td>
<td>0.1</td>
<td>0.09</td>
<td>90.2</td>
<td>19.09</td>
<td>0.12</td>
<td>8.5</td>
<td>19556.3</td>
</tr>
<tr>
<td>3</td>
<td>0.58</td>
<td>17.0</td>
<td>0.61</td>
<td>97.9</td>
<td>20.71</td>
<td>0.64</td>
<td>31.6</td>
<td>2716.5</td>
</tr>
<tr>
<td>4</td>
<td>0.70</td>
<td>29.1</td>
<td>0.72</td>
<td>39.6</td>
<td>8.39</td>
<td>0.74</td>
<td>8.1</td>
<td>955.9</td>
</tr>
<tr>
<td>5</td>
<td>0.90</td>
<td>0.4</td>
<td>0.97</td>
<td>54.4</td>
<td>11.52</td>
<td>1.00</td>
<td>24.0</td>
<td>2327.5</td>
</tr>
</tbody>
</table>

Total height = 472.5 total area = 9013.0
### Analysis e: Track 5

<table>
<thead>
<tr>
<th>Peak</th>
<th>Start Rf</th>
<th>Start H</th>
<th>Max Rf</th>
<th>Max H [%]</th>
<th>End Rf</th>
<th>End H</th>
<th>Area [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.00</td>
<td>3.6</td>
<td>0.01</td>
<td>599.6</td>
<td>0.04</td>
<td>158.0</td>
<td>1658.0</td>
</tr>
<tr>
<td>2</td>
<td>0.04</td>
<td>158.0</td>
<td>0.06</td>
<td>189.7</td>
<td>0.10</td>
<td>62.1</td>
<td>5609.3</td>
</tr>
<tr>
<td>3</td>
<td>0.10</td>
<td>62.1</td>
<td>0.11</td>
<td>69.5</td>
<td>0.16</td>
<td>29.2</td>
<td>2134.4</td>
</tr>
<tr>
<td>4</td>
<td>0.23</td>
<td>16.5</td>
<td>0.26</td>
<td>48.2</td>
<td>0.28</td>
<td>27.7</td>
<td>1255.1</td>
</tr>
<tr>
<td>5</td>
<td>0.28</td>
<td>27.7</td>
<td>0.29</td>
<td>34.6</td>
<td>0.32</td>
<td>1.4</td>
<td>575.6</td>
</tr>
<tr>
<td>6</td>
<td>0.34</td>
<td>3.6</td>
<td>0.36</td>
<td>26.6</td>
<td>0.39</td>
<td>15.0</td>
<td>601.3</td>
</tr>
<tr>
<td>7</td>
<td>0.39</td>
<td>17.9</td>
<td>0.41</td>
<td>36.8</td>
<td>0.43</td>
<td>21.1</td>
<td>1017.3</td>
</tr>
<tr>
<td>8</td>
<td>0.48</td>
<td>21.4</td>
<td>0.49</td>
<td>45.9</td>
<td>0.50</td>
<td>29.6</td>
<td>697.5</td>
</tr>
<tr>
<td>9</td>
<td>0.53</td>
<td>22.5</td>
<td>0.54</td>
<td>45.9</td>
<td>0.57</td>
<td>19.7</td>
<td>1225.2</td>
</tr>
<tr>
<td>10</td>
<td>0.60</td>
<td>37.7</td>
<td>0.62</td>
<td>56.7</td>
<td>0.63</td>
<td>56.8</td>
<td>1216.7</td>
</tr>
<tr>
<td>11</td>
<td>0.63</td>
<td>54.8</td>
<td>0.64</td>
<td>73.0</td>
<td>0.66</td>
<td>32.1</td>
<td>1131.6</td>
</tr>
<tr>
<td>12</td>
<td>0.69</td>
<td>40.8</td>
<td>0.72</td>
<td>139.8</td>
<td>0.76</td>
<td>8.9</td>
<td>3724.8</td>
</tr>
<tr>
<td>13</td>
<td>0.82</td>
<td>24.9</td>
<td>0.83</td>
<td>30.3</td>
<td>0.84</td>
<td>9.3</td>
<td>420.0</td>
</tr>
<tr>
<td>14</td>
<td>0.91</td>
<td>13.5</td>
<td>0.97</td>
<td>261.3</td>
<td>0.99</td>
<td>124.2</td>
<td>8267.3</td>
</tr>
</tbody>
</table>

**Total height = 1658.0**  
**Total area = 38694.9**

### Analysis f: Track 6

<table>
<thead>
<tr>
<th>Peak</th>
<th>Start Rf</th>
<th>Start H</th>
<th>Max Rf</th>
<th>Max H [%]</th>
<th>End Rf</th>
<th>End H</th>
<th>Area [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.00</td>
<td>3.6</td>
<td>0.01</td>
<td>599.6</td>
<td>0.04</td>
<td>158.0</td>
<td>1658.0</td>
</tr>
<tr>
<td>2</td>
<td>0.04</td>
<td>158.0</td>
<td>0.06</td>
<td>189.7</td>
<td>0.10</td>
<td>62.1</td>
<td>5609.3</td>
</tr>
<tr>
<td>3</td>
<td>0.10</td>
<td>62.1</td>
<td>0.11</td>
<td>69.5</td>
<td>0.16</td>
<td>29.2</td>
<td>2134.4</td>
</tr>
<tr>
<td>4</td>
<td>0.23</td>
<td>16.5</td>
<td>0.26</td>
<td>48.2</td>
<td>0.28</td>
<td>27.7</td>
<td>1255.1</td>
</tr>
<tr>
<td>5</td>
<td>0.28</td>
<td>27.7</td>
<td>0.29</td>
<td>34.6</td>
<td>0.32</td>
<td>1.4</td>
<td>575.6</td>
</tr>
<tr>
<td>6</td>
<td>0.34</td>
<td>3.6</td>
<td>0.36</td>
<td>26.6</td>
<td>0.39</td>
<td>15.0</td>
<td>601.3</td>
</tr>
<tr>
<td>7</td>
<td>0.39</td>
<td>17.9</td>
<td>0.41</td>
<td>36.8</td>
<td>0.43</td>
<td>21.1</td>
<td>1017.3</td>
</tr>
<tr>
<td>8</td>
<td>0.48</td>
<td>21.4</td>
<td>0.49</td>
<td>45.9</td>
<td>0.50</td>
<td>29.6</td>
<td>697.5</td>
</tr>
<tr>
<td>9</td>
<td>0.53</td>
<td>22.5</td>
<td>0.54</td>
<td>45.9</td>
<td>0.57</td>
<td>19.7</td>
<td>1225.2</td>
</tr>
<tr>
<td>10</td>
<td>0.60</td>
<td>37.7</td>
<td>0.62</td>
<td>56.7</td>
<td>0.63</td>
<td>56.8</td>
<td>1216.7</td>
</tr>
<tr>
<td>11</td>
<td>0.63</td>
<td>54.8</td>
<td>0.64</td>
<td>73.0</td>
<td>0.66</td>
<td>32.1</td>
<td>1131.6</td>
</tr>
<tr>
<td>12</td>
<td>0.69</td>
<td>40.8</td>
<td>0.72</td>
<td>139.8</td>
<td>0.76</td>
<td>8.9</td>
<td>3724.8</td>
</tr>
<tr>
<td>13</td>
<td>0.82</td>
<td>24.9</td>
<td>0.83</td>
<td>30.3</td>
<td>0.84</td>
<td>9.3</td>
<td>420.0</td>
</tr>
<tr>
<td>14</td>
<td>0.91</td>
<td>13.5</td>
<td>0.97</td>
<td>261.3</td>
<td>0.99</td>
<td>124.2</td>
<td>8267.3</td>
</tr>
</tbody>
</table>

**Total height = 1658.0**  
**Total area = 38694.9**
**Track 7, Analysis g: Track 7**

<table>
<thead>
<tr>
<th>Peak</th>
<th>Start</th>
<th>Max</th>
<th>End</th>
<th>Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.00</td>
<td>1.9</td>
<td>0.02</td>
<td>808.9</td>
</tr>
<tr>
<td>2</td>
<td>0.14</td>
<td>386.6</td>
<td>0.16</td>
<td>439.4</td>
</tr>
<tr>
<td>3</td>
<td>0.29</td>
<td>293.9</td>
<td>0.35</td>
<td>423.7</td>
</tr>
<tr>
<td>4</td>
<td>0.43</td>
<td>124.3</td>
<td>0.48</td>
<td>596.4</td>
</tr>
<tr>
<td>5</td>
<td>0.53</td>
<td>120.0</td>
<td>0.54</td>
<td>130.0</td>
</tr>
<tr>
<td>6</td>
<td>0.60</td>
<td>39.7</td>
<td>0.63</td>
<td>151.9</td>
</tr>
<tr>
<td>7</td>
<td>0.69</td>
<td>18.1</td>
<td>0.72</td>
<td>64.0</td>
</tr>
<tr>
<td>8</td>
<td>0.81</td>
<td>19.8</td>
<td>0.83</td>
<td>82.2</td>
</tr>
<tr>
<td>9</td>
<td>0.94</td>
<td>10.2</td>
<td>0.98</td>
<td>43.3</td>
</tr>
</tbody>
</table>

Total height = 2741.8  
Total area = 158873.1

**Track 8, Analysis h: Track 8**

<table>
<thead>
<tr>
<th>Peak</th>
<th>Start</th>
<th>Max</th>
<th>End</th>
<th>Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.01</td>
<td>91.0</td>
<td>0.03</td>
<td>125.7</td>
</tr>
<tr>
<td>2</td>
<td>0.04</td>
<td>80.7</td>
<td>0.08</td>
<td>143.5</td>
</tr>
<tr>
<td>3</td>
<td>0.13</td>
<td>1.0</td>
<td>0.16</td>
<td>74.1</td>
</tr>
<tr>
<td>4</td>
<td>0.18</td>
<td>33.2</td>
<td>0.19</td>
<td>45.9</td>
</tr>
<tr>
<td>5</td>
<td>0.24</td>
<td>2.8</td>
<td>0.27</td>
<td>42.5</td>
</tr>
<tr>
<td>6</td>
<td>0.29</td>
<td>37.2</td>
<td>0.35</td>
<td>229.3</td>
</tr>
<tr>
<td>7</td>
<td>0.43</td>
<td>0.4</td>
<td>0.49</td>
<td>503.4</td>
</tr>
<tr>
<td>8</td>
<td>0.53</td>
<td>39.0</td>
<td>0.54</td>
<td>71.0</td>
</tr>
<tr>
<td>9</td>
<td>0.59</td>
<td>0.3</td>
<td>0.63</td>
<td>114.6</td>
</tr>
<tr>
<td>10</td>
<td>0.69</td>
<td>5.2</td>
<td>0.72</td>
<td>50.3</td>
</tr>
<tr>
<td>11</td>
<td>0.81</td>
<td>19.6</td>
<td>0.84</td>
<td>85.8</td>
</tr>
<tr>
<td>12</td>
<td>0.94</td>
<td>5.3</td>
<td>0.97</td>
<td>34.5</td>
</tr>
</tbody>
</table>

Total height = 1521.6  
Total area = 52796.5
Track 9, Analysis i: Track 9

Peak start max end area

<table>
<thead>
<tr>
<th>#</th>
<th>Rf</th>
<th>H</th>
<th>Rf</th>
<th>H</th>
<th>H [%]</th>
<th>Rf</th>
<th>H</th>
<th>F</th>
<th>area [ %]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.00</td>
<td>34.2</td>
<td>0.03</td>
<td>195.3</td>
<td>15.46</td>
<td>0.05</td>
<td>26.7</td>
<td>4504.3</td>
<td>11.05</td>
</tr>
<tr>
<td>2</td>
<td>0.05</td>
<td>26.7</td>
<td>0.09</td>
<td>122.8</td>
<td>9.72</td>
<td>0.12</td>
<td>0.2</td>
<td>4293.7</td>
<td>10.53</td>
</tr>
<tr>
<td>3</td>
<td>0.13</td>
<td>0.4</td>
<td>0.16</td>
<td>66.3</td>
<td>5.25</td>
<td>0.19</td>
<td>16.7</td>
<td>1436.9</td>
<td>3.52</td>
</tr>
<tr>
<td>4</td>
<td>0.19</td>
<td>16.7</td>
<td>0.20</td>
<td>26.9</td>
<td>2.13</td>
<td>0.23</td>
<td>7.4</td>
<td>592.3</td>
<td>1.45</td>
</tr>
<tr>
<td>5</td>
<td>0.30</td>
<td>1.5</td>
<td>0.37</td>
<td>193.1</td>
<td>15.29</td>
<td>0.44</td>
<td>0.7</td>
<td>11233.8</td>
<td>27.56</td>
</tr>
<tr>
<td>6</td>
<td>0.44</td>
<td>0.1</td>
<td>0.49</td>
<td>387.4</td>
<td>30.67</td>
<td>0.53</td>
<td>22.2</td>
<td>12253.1</td>
<td>30.06</td>
</tr>
<tr>
<td>7</td>
<td>0.54</td>
<td>22.8</td>
<td>0.55</td>
<td>38.0</td>
<td>3.01</td>
<td>0.59</td>
<td>0.6</td>
<td>726.7</td>
<td>1.78</td>
</tr>
<tr>
<td>8</td>
<td>0.59</td>
<td>1.5</td>
<td>0.63</td>
<td>48.3</td>
<td>3.83</td>
<td>0.67</td>
<td>2.6</td>
<td>1367.4</td>
<td>3.35</td>
</tr>
<tr>
<td>9</td>
<td>0.69</td>
<td>4.3</td>
<td>0.73</td>
<td>55.1</td>
<td>4.35</td>
<td>0.76</td>
<td>1.6</td>
<td>1421.6</td>
<td>3.49</td>
</tr>
<tr>
<td>10</td>
<td>0.77</td>
<td>1.6</td>
<td>0.79</td>
<td>24.4</td>
<td>1.94</td>
<td>0.80</td>
<td>16.0</td>
<td>406.7</td>
<td>1.00</td>
</tr>
<tr>
<td>11</td>
<td>0.82</td>
<td>17.4</td>
<td>0.84</td>
<td>31.1</td>
<td>2.46</td>
<td>0.87</td>
<td>0.3</td>
<td>764.3</td>
<td>1.88</td>
</tr>
<tr>
<td>12</td>
<td>0.91</td>
<td>0.3</td>
<td>0.93</td>
<td>22.6</td>
<td>1.79</td>
<td>0.93</td>
<td>9.7</td>
<td>159.3</td>
<td>0.39</td>
</tr>
<tr>
<td>13</td>
<td>0.93</td>
<td>10.4</td>
<td>0.97</td>
<td>51.6</td>
<td>4.09</td>
<td>1.00</td>
<td>17.4</td>
<td>1602.8</td>
<td>3.93</td>
</tr>
</tbody>
</table>

Total height = 1262.9
Total area = 40762.8

---

Analysis j: Gallic Acid

Wavelength: 254 nm

Track 10, Analysis j: Gallic Acid

Peak start max end area

<table>
<thead>
<tr>
<th>#</th>
<th>Rf</th>
<th>H</th>
<th>Rf</th>
<th>H</th>
<th>H [%]</th>
<th>Rf</th>
<th>H</th>
<th>F</th>
<th>area [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.46</td>
<td>11.7</td>
<td>0.52</td>
<td>459.6</td>
<td>100.00</td>
<td>0.56</td>
<td>3.5</td>
<td>14768.5</td>
<td>100.00</td>
</tr>
</tbody>
</table>

Total height = 459.6
Total area = 14768.5
Triphala formulations standardized by HPTLC method using gallic acid as marker compound. The plate was developed in Toluene: Ethyl acetate: Formic acid (5:5:1) solvent system. Gallic acid was traced at 0.52 Rf value (track-10), when scanned at 254 nm. Triphala equal showed major peak at 0.48 Rf (track-7), Triphala unequal (track-8) and Chinnodbhavadi kwath (track-9) showed peak at 0.49 Rf value when scanned at 254 nm. The Rf values for gallic acid in all three Triphala formulations almost near to the values observed in standard gallic acid compound.
3. PHARMACOLOGICAL STUDIES

3.1. GASTRO-CYTOPROTECTIVE ACTIVITY

Gastro-protective activity of Triphala equal, Triphala unequal, Chinnodbhavadi kwath carried out by using aspirin administration plus pyloric ligation - induced gastric ulcer and 14 h water immersion stress - induced gastric ulcer in albino rats

3.1.1. Gastric ulcers- induced by aspirin plus pyloric ligation in albino rats.

Aspirin in dose of 200 mg/kg for 3 days plus pyloric ligation for 6 hours resulted in to gastric ulcers in rats. The drugs were studied for its effect on secretory parameters, total protein, total carbohydrates, mucin activity in gastric juice and nucleic acid content in stomach homogenate of rats.

Table-5
Effect of Triphala formulations on gastric juice parameters in aspirin plus pyloric ligated albino rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Volume of gastric fluid (ml/100g BW)</th>
<th>pH of gastric fluid</th>
<th>Free acidity (mEq HCl/100 ml)</th>
<th>Total acidity (mEq HCl/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.00 ± 0.09</td>
<td>3.46 ± 0.16</td>
<td>13.66 ± 1.58</td>
<td>108.00 ± 4.90</td>
</tr>
<tr>
<td>Triphala equal</td>
<td>1.94 ± 0.09</td>
<td>3.86 ± 0.10</td>
<td>10.66 ± 1.52</td>
<td>94.00 ± 3.50 **</td>
</tr>
<tr>
<td>Triphala unequal</td>
<td>1.86 ± 0.10</td>
<td>3.81 ± 0.14</td>
<td>08.33 ± 0.80 a</td>
<td>93.66 ± 3.11 **</td>
</tr>
<tr>
<td>Chinnod. Kwath</td>
<td>1.84 ± 0.05</td>
<td>3.88 ± 0.17</td>
<td>06.33 ± 0.61 b</td>
<td>87.33 ± 2.66 b</td>
</tr>
<tr>
<td>Omeprazole</td>
<td>1.69 ± 0.04 *</td>
<td>4.70 ± 0.13 b</td>
<td>04.66 ± 0.66 b</td>
<td>83.16 ± 0.83 b</td>
</tr>
</tbody>
</table>

The values are expressed as Mean ± SEM
* P<0.05, ** P<0.02, a P<0.01, b P<0.001 compared with control group.

Pylorus ligation for 6 h plus aspirin administration resulted in accumulation of gastric secretory volume, decrease in pH and increase in titrable acidity (table-5). Pretreatment with omeprazole significantly reduced volume of gastric juice as it is an anti-secretory agent and increased pH significantly in comparison to control group. However, the weak to moderate decrease in volume of gastric contents and increase in pH did not reach statistically significant level with that of Triphala formulations treated groups.
Oral administration of Triphala unequal, kwath and omeprazole significantly reduced the free acidity. All the three Triphala formulations significantly reduced the total acidity in comparison to control group and results obtained were comparable with the standard group. From the presented data, it is observed that antacid effect of kwath was more pronounced followed by Triphala unequal and Triphala equal when compared with the control group.

Table-6

Effect of Triphala formulations on the level of dissolved mucosubstances in gastric juice in aspirin plus pyloric ligated albino rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total hexose (µg/ml)</th>
<th>Total fucose (µg/ml)</th>
<th>Hexosamine (µg/ml)</th>
<th>Sialic acid (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>365.87 ± 07.31</td>
<td>092.59 ± 6.32</td>
<td>209.87 ± 26.47</td>
<td>28.88 ± 4.00</td>
</tr>
<tr>
<td>Triphala equal</td>
<td>359.14 ± 16.55</td>
<td>110.54 ± 7.71</td>
<td>216.04 ± 20.09</td>
<td>35.55 ± 7.23</td>
</tr>
<tr>
<td>Triphala unequal</td>
<td>428.72 ± 25.65</td>
<td>105.82 ± 4.05</td>
<td>271.60 ± 26.47</td>
<td>36.11 ± 5.60</td>
</tr>
<tr>
<td>Chinnod. Kwath</td>
<td>534.23 ± 32.18 **</td>
<td>142.66 ± 3.98 **</td>
<td>253.08 ± 26.04</td>
<td>36.66 ± 5.71</td>
</tr>
<tr>
<td>Omeprazole</td>
<td>493.82 ± 13.31 **</td>
<td>102.04 ± 3.87</td>
<td>320.98 ± 31.23 *</td>
<td>41.10 ± 3.61</td>
</tr>
</tbody>
</table>

The values are expressed as Mean ± SEM

* P< 0.05, ** P< 0.001, compared with control group.

The level of dissolved mucosubstances had been studied by estimating individual carbohydrate constituents and protein content in the alcoholic precipitates of gastric juice. The data related to the concentration of individual carbohydrate constituents such as total hexose, total fucose, hexosamine and sialic acid are presented in the (table-6). The level of total hexose was significantly increased in kwath and omeprazole treated groups, though an apparent increase was observed in other test drugs administered group but that was not statistically significant. In the Chinnodbhavadi kwath treated group, the level of total hexose significantly increased at level of P< 0.001. The increased level of total hexose and fucose observed in kwath treated group were comparable with standard group.
Table-7

Effect of Triphala formulations on total carbohydrate, total protein, TC:TP ratio and pepsin activity in gastric juice in aspirin plus pyloric ligated albino rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total carbohydrate (μg/ml)</th>
<th>Total protein (μg/ml)</th>
<th>TC:TP ratio</th>
<th>Pepsin activity (μmoles of tyrosine released/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>697.29 ± 31.71</td>
<td>679.72 ± 33.31</td>
<td>1.027 ± 0.022</td>
<td>462.82 ± 14.28</td>
</tr>
<tr>
<td>Triphala equal</td>
<td>721.28 ± 32.59</td>
<td>661.42 ± 40.99</td>
<td>1.112 ± 0.068</td>
<td>423.31 ± 25.86</td>
</tr>
<tr>
<td>Triphala unequal</td>
<td>842.25 ± 35.67 *</td>
<td>674.49 ± 26.86</td>
<td>1.253 ± 0.046 **</td>
<td>412.02 ± 32.03</td>
</tr>
<tr>
<td>Chinnod. Kwath</td>
<td>966.64 ± 43.31 ***</td>
<td>666.65 ± 18.88</td>
<td>1.450 ± 0.050 ***</td>
<td>423.31 ± 22.71</td>
</tr>
<tr>
<td>Omeprazole</td>
<td>957.95 ± 39.88 ***</td>
<td>671.88 ± 34.94</td>
<td>1.435 ± 0.060 ***</td>
<td>372.51 ± 24.73</td>
</tr>
</tbody>
</table>

The values are expressed as Mean ± SEM
* P< 0.05, ** P<0.02, *** P< 0.001, compared with control group.

Total carbohydrate values represents the sum of total hexose, fucose, hexosamine and sialic acid in gastric juice of individual rat (table-7). Chinnodbhavadi kwath significantly increased the level of total carbohydrate level at level of P< 0.001 which was comparable with the values obtained in standard group. Triphala equal and Triphala unequal also increased the level of carbohydrate content but only Triphala unequal group showed significant increase in carbohydrate at P< 0.05. All the three formulations showed a little or no effect on total protein content in the alcoholic precipitates of gastric juice.

Total carbohydrate to total protein (TC:TP) ratio is taken as reliable index for mucin activity (Sanyal et al., 1983). Triphala unequal and kwath showed significant increase in TC:TP ratio, indicate that formulations enhances mucin secretion which is one of the defensive mucosal factor, however mucin secretion values were not raised to significant level in Triphala equal pretreated group. Pepsin activity in the gastric juice which is considered as one of the offensive factors responsible for gastroduodenal ulceration in pyloric ligation plus aspirin administration in rats was not influenced by any of the Triphala formulations. A statistically significant decrease was however, observed in omeprazole treated group.
Table-8
Effect of Triphala formulations on tissue DNA and RNA in stomach tissue homogenate of albino rats with aspirin plus pyloric ligation - induced ulcer

<table>
<thead>
<tr>
<th>Groups</th>
<th>DNA (µg/ g tissue)</th>
<th>RNA (µg/ g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>510.86 ± 26.17</td>
<td>868.41 ± 78.46</td>
</tr>
<tr>
<td>Triphala equal</td>
<td>652.17 ± 37.65 *</td>
<td>992.47 ± 90.60</td>
</tr>
<tr>
<td>Triphala unequal</td>
<td>619.56 ± 36.70</td>
<td>992.48 ± 45.30</td>
</tr>
<tr>
<td>Chinnod. Kwath</td>
<td>663.04 ± 42.65 *</td>
<td>1054.51 ± 69.81</td>
</tr>
<tr>
<td>Omeprazole</td>
<td>652.17 ± 44.55 *</td>
<td>1054.51 ± 83.22</td>
</tr>
</tbody>
</table>

The values are expressed as Mean ± SEM
* P< 0.05 compared with control group.

The data related to the effect of Triphala formulations on DNA and RNA contents of the gastric wall mucosa in aspirin plus pyloric ligation - induced gastric ulceration in rats are shown in (table-8). Increase or decrease in life span of mucosal cells can be expressed as the amount of DNA and RNA in the gastric wall mucosa. It can be seen from the graph that DNA and RNA values observed in control groups were (510.86 ± 26.17) and (868.41 ± 78.46) respectively. The rats pretreated with all three Triphala formulations showed increase in the values of DNA and RNA, indicating the enhancement of life span of mucosal cells. However, increase in DNA content was found statistically significant (P<0.05) in Triphala equal and kwath treated groups only. Increase in RNA content in all treated groups was not found to be statistically significant.
**Fig.-7**

**Effect of Triphala formulations on ulcer index in aspirin plus pyloric ligation - induced ulcer in albino rats**

![Graph showing ulcer index for different groups](image)

The values are expressed as Mean ± SEM

* P< 0.05, ** P<0.01, *** P< 0.001, compared with control group.

Triphala unequal, Chinnodbhavadi kwath and omeprazole showed significant gastric ulcer protective effect as evident from the decreased intensity of gastric mucosal ulceration induced by aspirin plus pyloric ligation in rats (Fig.-7). The kwath produced more pronounced effect than Triphala powders as indicated by decreased the gastric ulcer index at significant level of P< 0.01 as compared to the result of Triphala unequal that is P< 0.05.
Photomicrograph of stomach tissue from pyloric ligation plus aspirin induced ulcer model

<table>
<thead>
<tr>
<th>Stomach from control group (1x100) (Severe epithelial destruction, cell infiltration in muscular layer)</th>
<th>Stomach from control group (1x100) (Severe epithelial destruction and oedematous changes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomach from control group (1x400) (Severe epithelial destruction)</td>
<td>Stomach from Triphala equal treated group (1x100) (Severe epithelial destruction)</td>
</tr>
<tr>
<td>Stomach from Triphala equal treated group (1x100) (epithelial layer undisturbed)</td>
<td>Stomach from Triphala unequal treated group (1x100) (epithelial layer almost normal)</td>
</tr>
</tbody>
</table>
Stomach from Triphala unequal treated group (1x100) (Epithelial layer almost normal)

Stomach from Triphala unequal treated group (1x400) (Almost normal cytoarchitecture)

Stomach from Chinnod. kwath treated group (1x100) (Epithelial layer almost normal)

Stomach from Chinnod. kwath treated group (1x100) (Mild epithelial disruption with cell infiltration)

Stomach from omeprazole treated group (1x100) (Almost normal cytoarchitecture)

Stomach from omeprazole treated group (1x400) (Almost normal cytoarchitecture)

El- Epithelial layer, SM- Submucosa, ML- Musculayer layer, SL- Serous layer
3.1.2. Stress - induced gastric ulcer and hypothermia

Rats were subjected to 14 h water immersion stress resulted in sever ulcer in albino rats. The effects of Triphala equal, Triphala unequal and Chinnodbhavadi kwath were studied for its effect on haematological parameters, biochemical parameters in serum and tissue.

Table-9
Effect of Triphala formulations on haematological parameters in albino rats subjected to water immersion stress

<table>
<thead>
<tr>
<th>Groups</th>
<th>WBC (10^3/μl)</th>
<th>Lymphocyte %</th>
<th>Monocytes %</th>
<th>Granulocyte %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>3.31 ± 0.19</td>
<td>83 ± 1.52</td>
<td>5.18 ± 0.71</td>
<td>11.88 ± 0.73</td>
</tr>
<tr>
<td>Stress control</td>
<td>1.57 ± 0.25 a</td>
<td>82 ± 2.45</td>
<td>4.00 ± 1.08</td>
<td>13.90 ± 1.66</td>
</tr>
<tr>
<td>Triphala equal</td>
<td>1.73 ± 0.41</td>
<td>84 ± 1.99</td>
<td>2.86 ± 0.28</td>
<td>12.55 ± 1.92</td>
</tr>
<tr>
<td>Triphala unequal</td>
<td>1.65 ± 0.20</td>
<td>75 ± 3.62</td>
<td>6.40 ± 0.97</td>
<td>15.11 ± 1.71</td>
</tr>
<tr>
<td>Chinnod. Kwath</td>
<td>2.00 ± 0.23</td>
<td>71.61 ± 1.07</td>
<td>8.15 ± 0.28</td>
<td>19.51 ± 1.09</td>
</tr>
<tr>
<td>Omeprazole</td>
<td>2.51 ± 0.59</td>
<td>68.98 ± 2.74</td>
<td>8.55 ± 0.85</td>
<td>23.03 ± 1.83</td>
</tr>
</tbody>
</table>

The values are expressed as Mean ± SEM

*P< 0.02, **P< 0.01, ***P< 0.001 compared with stress control group.

* P< 0.01 compared with normal control group.

Stress produced significant reduction in total WBC count while differential count was not affected in stress control in comparison to normal control group (table-9). Though an apparent reversal of this stress - induced decrease in WBC count observed in test drug treated groups; the reversal did not reach statistically significant levels. Though stress per se had no effect on lymphocyte and monocyte percentage; stressed rats pretreated with kwath and standard drug showed significant reduction in lymphocytes percentage and significant increase in monocyte percentage in comparison to stress control rats.
Table-10
Effect of Triphala formulations on haematological parameters in albino rats subjected to water immersion stress

<table>
<thead>
<tr>
<th>Groups</th>
<th>RBC (10⁶/μl)</th>
<th>MCV (μm³)</th>
<th>MCH (pg)</th>
<th>MCHC (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>11.69 ± 0.62</td>
<td>67.42 ± 1.44</td>
<td>12.46 ± 0.39</td>
<td>18.83 ± 0.59</td>
</tr>
<tr>
<td>Stress control</td>
<td>10.09 ± 0.60</td>
<td>69.25 ± 1.52</td>
<td>10.81 ± 0.73</td>
<td>16.75 ± 1.04</td>
</tr>
<tr>
<td>Triphala equal</td>
<td>11.08 ± 0.40</td>
<td>72.53 ± 0.60</td>
<td>11.28 ± 0.98</td>
<td>15.45 ± 1.38</td>
</tr>
<tr>
<td>Triphala unequal</td>
<td>11.00 ± 0.56</td>
<td>68.06 ± 0.99</td>
<td>13.51 ± 0.24 **</td>
<td>19.88 ± 0.31 *</td>
</tr>
<tr>
<td>Chinnod. Kwath</td>
<td>10.04 ± 0.60</td>
<td>67.53 ± 0.62</td>
<td>12.78 ± 0.12</td>
<td>19.00 ± 0.26</td>
</tr>
<tr>
<td>Omeprazole</td>
<td>11.64 ± 0.54</td>
<td>66.15 ± 1.21</td>
<td>12.60 ± 0.25</td>
<td>19.51 ± 0.21</td>
</tr>
</tbody>
</table>

The values are expressed as Mean ± SEM

* P< 0.05, ** P< 0.01 compared with stress control group.

Heparinized blood samples from the group mentioned above were used for estimation of RBC and related parameters (table-10). Triphala unequal significantly increased the value of mean cell haemoglobin content (MCH) (P< 0.05) and mean cell haemoglobin concentration (MCHC) (P< 0.01) as compared to stress control group. However, other parameters were not affected by the stress and test drug treatment in rats.

Table-11
Effect of Triphala formulations on haematological parameters in albino rats subjected to water immersion stress

<table>
<thead>
<tr>
<th>Groups</th>
<th>HCT (%)</th>
<th>RDW (%)</th>
<th>Haemoglobin (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>73.53 ± 1.93</td>
<td>7.11 ± 0.19</td>
<td>14.73 ± 0.29</td>
</tr>
<tr>
<td>Stress control</td>
<td>69.71 ± 3.81</td>
<td>6.71 ± 0.31</td>
<td>10.80 ± 0.95 a</td>
</tr>
<tr>
<td>Triphala equal</td>
<td>80.93 ± 2.79</td>
<td>6.66 ± 0.13</td>
<td>12.61 ± 1.44</td>
</tr>
<tr>
<td>Triphala unequal</td>
<td>75.05 ± 4.43</td>
<td>7.38 ± 0.11</td>
<td>14.85 ± 0.76 *</td>
</tr>
<tr>
<td>Chinnod. Kwath</td>
<td>67.21 ± 4.13</td>
<td>7.38 ± 0.14</td>
<td>12.73 ± 0.83</td>
</tr>
<tr>
<td>Omeprazole</td>
<td>75.51 ± 2.70</td>
<td>7.36 ± 0.21</td>
<td>12.71 ± 0.77</td>
</tr>
</tbody>
</table>

The values are expressed as Mean ± SEM

* P< 0.02 compared with stress control group.

a P< 0.02 compared with normal control group.
Table-11 contains data related to the effect of test drugs on HCT%, RDW% and hemoglobin content in different groups. It can be observed that stress significantly reduced the haemoglobin level in comparison to normal control group. In Triphala unequal group, there was significant (P<0.05) reversal of the stress - induced depletion of hemoglobin content. Though an apparent reversal could be seen in other test drug administered groups, the magnitude of reversal did not reach statistically significant level.

Table-12
Effect of Triphala formulations on serum biochemical parameters in albino rats subjected to water immersion stress

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total Protein (g/dl)</th>
<th>Albumin (g/dl)</th>
<th>Sugar (mg/dl)</th>
<th>Urea (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>8.57 ± 0.55</td>
<td>4.47 ± 0.26</td>
<td>98.50 ± 3.72</td>
<td>39.50 ± 2.89</td>
</tr>
<tr>
<td>Stress control</td>
<td>5.79 ± 0.55 **</td>
<td>2.93 ± 0.28 **</td>
<td>20.16 ± 3.96 **</td>
<td>67.33 ± 6.31 *</td>
</tr>
<tr>
<td>Triphala equal</td>
<td>6.26 ± 0.35</td>
<td>2.71 ± 0.32</td>
<td>24.33 ± 6.59</td>
<td>59.16 ± 6.51</td>
</tr>
<tr>
<td>Triphala unequal</td>
<td>5.55 ± 0.36</td>
<td>3.64 ± 0.21</td>
<td>17.33 ± 1.62</td>
<td>58.33 ± 5.65</td>
</tr>
<tr>
<td>Chinnod. Kwath</td>
<td>6.26 ± 0.31</td>
<td>3.65 ± 0.12</td>
<td>29.00 ± 4.67</td>
<td>52.83 ± 3.68</td>
</tr>
<tr>
<td>Omeprazole</td>
<td>6.82 ± 0.48</td>
<td>4.25 ± 0.28 *</td>
<td>15.50 ± 1.74</td>
<td>42.33 ± 4.01 *</td>
</tr>
</tbody>
</table>

The values are expressed as Mean ± SEM
* P< 0.01 compared with stress control group.
** P< 0.001 compared with normal control group.

Water immersion stress for 14 h produced marked reduction in total protein, albumin, sugar and elevated urea level in the serum when compared with the normal control rats. Pretreatment with omeprazole significantly restored the depleted level of albumin and suppressed stress - induced elevation in urea level with no remarkable changes produced in total protein and sugar level in serum (table-12). Pretreatment with all Triphala formulations did not show significant alteration in those parameters as compared to stress control group.
Table-13
Effect of Triphala formulations on serum biochemical parameters in albino rats subjected to water immersion stress

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total Cholesterol (mg/dl)</th>
<th>HDL Cholesterol (mg/dl)</th>
<th>Triglycerides (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>67.00 ± 0.41</td>
<td>61.83 ± 5.19</td>
<td>37.00 ± 2.97</td>
</tr>
<tr>
<td>Stress control</td>
<td>69.00 ± 10.21</td>
<td>47.33 ± 8.61</td>
<td>24.66 ± 2.68 *</td>
</tr>
<tr>
<td>Triphala equal</td>
<td>91.00 ± 06.44</td>
<td>58.33 ± 6.45</td>
<td>25.83 ± 2.73</td>
</tr>
<tr>
<td>Triphala unequal</td>
<td>81.50 ± 12.23</td>
<td>56.33 ± 10.46</td>
<td>27.50 ± 3.16</td>
</tr>
<tr>
<td>Chinnod. Kwath</td>
<td>70.83 ± 04.07</td>
<td>40.66 ± 3.58</td>
<td>28.33 ± 2.49</td>
</tr>
<tr>
<td>Omeprazole</td>
<td>83.50 ± 06.89</td>
<td>58.16 ± 4.16</td>
<td>31.50 ± 0.80</td>
</tr>
</tbody>
</table>

The values are expressed as Mean ± SEM
* P< 0.05 compared with normal control group.

As could be observed (table-13), water immersion stress did not affect serum cholesterol level to significant extent. Though an apparent moderate decrease was observed in HDL-cholesterol level, it was found to be statistically non-significant. In contrast to the above, a statistically significant decrease in serum triglyceride level was observed in stressed rats in comparison to non-stressed normal rats. However, none of Triphala formulations and standard drug altered the lipid profile significantly in rats subjected to stress.

Table-14
Effect of Triphala formulations on serum biochemical parameters in albino rats subjected to water immersion stress

<table>
<thead>
<tr>
<th>Groups</th>
<th>Alkaline Phosphatase (IU/L)</th>
<th>SGPT (IU/L)</th>
<th>SGOT (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>087.33 ± 04.97</td>
<td>119.16 ± 07.84</td>
<td>299.50 ± 27.17</td>
</tr>
<tr>
<td>Stress control</td>
<td>280.66 ± 18.46 ***</td>
<td>179.33 ± 12.44 **</td>
<td>390.66 ± 49.57</td>
</tr>
<tr>
<td>Triphala equal</td>
<td>284.66 ± 30.18</td>
<td>162.00 ± 19.69</td>
<td>417.16 ± 23 84</td>
</tr>
<tr>
<td>Triphala unequal</td>
<td>201.00 ± 20.07 *</td>
<td>122.33 ± 10.90 *</td>
<td>384.33 ± 31 04</td>
</tr>
<tr>
<td>Chinnod. Kwath</td>
<td>227.00 ± 18.35</td>
<td>141.16 ± 14.12</td>
<td>378.33 ± 33 64</td>
</tr>
<tr>
<td>Omeprazole</td>
<td>199.00 ± 19.79 *</td>
<td>152.50 ± 20.76</td>
<td>330.83 ± 16.87</td>
</tr>
</tbody>
</table>

The values are expressed as Mean ± SEM
* P< 0.05 compared with stress control group.
** P< 0.05, *** P< 0.001 compared with normal control group.
Results and Observations

Serum level of alkaline phosphatase and SGPT in rats subjected to water immersion stress were significantly higher than the corresponding values in control group (table-14). A moderate but statistically non-significant elevation was observed in SGOT activity. The increased activity of alkaline phosphatase was found to be significantly attenuated by the pre-treatment with Triphala unequal and omeprazole in comparison to stress control group (201.00 ± 20.07 and 199.00 ± 19.78 v/s 280.66 ± 18.46). None of the drugs could influence the SGOT activity to significant extent. However, Triphala unequal produced significant decrease in SGPT at a level of P< 0.05.

Table-15

Effect of Triphala formulations on biochemical parameters in gastric homogenate in albino rats subjected to water immersion stress

<table>
<thead>
<tr>
<th>Groups</th>
<th>Lipid Peroxidation (n moles of MDA formed/g tissue)</th>
<th>Total protein (mg/g tissue)</th>
<th>Hydroxy radical (Unit/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>5.04 ± 0.57</td>
<td>92.66 ± 4.12</td>
<td>4.52 ± 0.49</td>
</tr>
<tr>
<td>Stress control</td>
<td>8.38 ± 0.53(^b)</td>
<td>86.90 ± 4.83</td>
<td>9.36 ± 0.58(^b)</td>
</tr>
<tr>
<td>Triphala equal</td>
<td>7.36 ± 0.29</td>
<td>89.77 ± 3.56</td>
<td>7.84 ± 0.24</td>
</tr>
<tr>
<td>Triphala unequal</td>
<td>6.68 ± 0.25(^*)</td>
<td>85.75 ± 3.82</td>
<td>7.59 ± 0.32(^*)</td>
</tr>
<tr>
<td>Chinnod. Kwath</td>
<td>6.68 ± 0.39(^*)</td>
<td>82.87 ± 7.87</td>
<td>7.37 ± 0.31(^**)</td>
</tr>
<tr>
<td>Omeprazole</td>
<td>6.13 ± 0.55(^***)</td>
<td>87.47 ± 4.93</td>
<td>5.53 ± 0.53(^a)</td>
</tr>
</tbody>
</table>

The values are expressed as Mean ± SEM

\(^*\)P< 0.05; \(^**\)P< 0.02; \(^***\)P< 0.01; \(^a\)P< 0.001 compared with stress control group.

Water immersion stress produced significant elevation in the lipid peroxidation and hydroxyl radicals release in gastric homogenates in comparison to non-stressed control group (table-15). Stressed animals pretreated with Triphala unequal, Chinnod. kwath and standard drug, reversed the stress-induced changes observed in lipid peroxidation and hydroxyl radical values which were near to normal control values. In Triphala equal group, only a moderate and statistically non-significant reduction was observed in LPO and hydroxyl radicals.

Moderate and non significant decrease in protein content was observed in stress control group in comparison to normal control, which shows increased leakage.
of gastric mucosal protein in to gastric juice in stress - induced ulcer. Test drugs and reference standard did not influence the total protein content to significant extent in gastric mucosa of stressed rats.

Table-16
Effect of Triphala formulations on biochemical parameters in gastric homogenate in albino rats subjected to water immersion stress

<table>
<thead>
<tr>
<th>Groups</th>
<th>Superoxide Dismutase (Unit/mg protein)</th>
<th>Catalase (µmoles H₂O₂ consumed/mg protein/min)</th>
<th>Total Glutathione (nmoles/g tissue)</th>
<th>GPx (µg of GSH utilized/mg protein/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>1.26 ± 0.04</td>
<td>7.80 ± 0.31</td>
<td>1418.36 ± 48.17</td>
<td>7.14 ± 0.42</td>
</tr>
<tr>
<td>Stress control</td>
<td>1.06 ± 0.05 &lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.39 ± 0.26 &lt;sup&gt;a&lt;/sup&gt;</td>
<td>0618.79 ± 58.42 &lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.87 ± 0.33</td>
</tr>
<tr>
<td>Triphala equal</td>
<td>1.07 ± 0.05</td>
<td>6.97 ± 0.21</td>
<td>0813.47 ± 47.87 &lt;sup&gt;*&lt;/sup&gt;</td>
<td>6.59 ± 0.41</td>
</tr>
<tr>
<td>Triphala unequal</td>
<td>1.24 ± 0.04 &lt;sup&gt;*&lt;/sup&gt;</td>
<td>7.83 ± 0.31 &lt;sup&gt;*&lt;/sup&gt;</td>
<td>0881.37 ± 45.27</td>
<td>7.15 ± 0.24</td>
</tr>
<tr>
<td>Chinnod. Kwhath</td>
<td>1.24 ± 0.06 &lt;sup&gt;*&lt;/sup&gt;</td>
<td>7.91 ± 0.55 &lt;sup&gt;*&lt;/sup&gt;</td>
<td>0889.93 ± 55.61 &lt;sup&gt;**&lt;/sup&gt;</td>
<td>6.79 ± 0.57</td>
</tr>
<tr>
<td>Omeprazole</td>
<td>1.19 ± 0.03</td>
<td>7.28 ± 0.44</td>
<td>1035.94 ± 48.66 &lt;sup&gt;***&lt;/sup&gt;</td>
<td>6.72 ± 0.37</td>
</tr>
</tbody>
</table>

The values are expressed as Mean ± SEM
*P< 0.05, **P< 0.01, ***P< 0.001 compared with stress control group.
<sup>a</sup> P< 0.05, <sup>b</sup> P< 0.001 compared with normal control group.

The data pertaining to the effect of Triphala formulations on anti-oxidants level in gastric homogenates obtained from rats subjected to water immersion stress have been shown in (table-16). Stress significantly reduced the activity of superoxide dismutase (P< 0.05), catalase (P< 0.05) and total glutathione (P< 0.001) content in gastric mucosa of rats when compared with the normal control group. Superoxide dismutase and catalase level observed in Triphala unequal (1.24 ± 0.04 and 7.83 ± 0.30) and kwath treated group (1 24 ± 0.06 and 7.91 ± 0.55) were found near to the values observed in normal control group (1 26 ± 0.04 and 7.80 ± 0.31) and found statistically significant at P< 0.05, when compared with the stress control group.

Cellular antioxidant levels such as glutathione was significantly raised in Triphala equal and kwath treated groups when compared with the stress control group. However, the level of glutathione peroxidase, which considered as first line of defense, was not altered to significant extent by stress and also not influenced by pre-treatment of drugs in stress.
Table-17

Effect of Triphala formulations on biochemical parameters in gastric homogenate in albino rats subjected to water immersion stress

<table>
<thead>
<tr>
<th>Groups</th>
<th>Na-ATPase (µmoles of phosphorus liberated /mg protein/min)</th>
<th>Ca-ATPase (µmoles of phosphorus liberated /mg protein/min)</th>
<th>Mg-ATPase (µmoles of phosphorus liberated /mg protein/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>3.53 ± 0.19</td>
<td>2.62 ± 0.14</td>
<td>2.49 ± 0.16</td>
</tr>
<tr>
<td>Stress control</td>
<td>2.16 ± 0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.28 ± 0.15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.35 ± 0.21&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Triphala equal</td>
<td>2.38 ± 0.23</td>
<td>0.98 ± 0.10</td>
<td>1.09 ± 0.07</td>
</tr>
<tr>
<td>Triphala unequal</td>
<td>2.67 ± 0.18</td>
<td>1.84 ± 0.15</td>
<td>1.60 ± 0.13</td>
</tr>
<tr>
<td>Chinnod. Kwath</td>
<td>3.81 ± 0.53&lt;sup&gt;***&lt;/sup&gt;</td>
<td>2.34 ± 0.23&lt;sup&gt;****&lt;/sup&gt;</td>
<td>2.01 ± 0.16&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>Omeprazole</td>
<td>3.38 ± 0.47&lt;sup&gt;*&lt;/sup&gt;</td>
<td>2.14 ± 0.12&lt;sup&gt;***&lt;/sup&gt;</td>
<td>1.94 ± 0.06&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

The values are expressed as Mean ± SEM

*P< 0.05, **P< 0.02, ***P< 0.01, ****P< 0.001 compared with stress control group.

<sup>a</sup>P< 0.05, <sup>b</sup>P< 0.001 compared with normal control group

The data related to the effect of Triphala formulations on membrane bound ATPases level in gastric mucosa are summarized in (table-17). In rats subjected to water immersion stress, there was significant decrease in the level of membrane bound Na-ATPase (P< 0.05), Ca-ATPase and Mg-ATPase (P< 0.001) in comparison to normal control group. Kwath significantly reversed the decreased level of membrane bound ATPase near to normal control group in stress condition. Triphala equal and unequal had no significant effect on the membrane bound ATPase activity in comparison to stress control group.
Results and Observations

Fig.-8
Effect of Triphala formulations on ulcer index in stomach of albino rats subjected to water immersion stress

![Graph showing ulcer index with mean ± SEM values.](image)

The values are expressed as Mean ± SEM
*P< 0.05, ** P< 0.02, *** P< 0.001 compared with stress control group.

Fig.-9
Effect of Triphala formulations on hypothermia in albino rats subjected to water immersion stress for 15 min.

![Graph showing hypothermia with mean ± SEM values.](image)

The values are expressed as Mean ± SEM
*P< 0.05, ** P< 0.01 compared with stress control group.

Rats subjected to water immersion stress showed significant increase in intensity of gastric ulcers as shown in (Fig.-8). The rats pretreated with three Triphala formulations and omeprazole reduced the severity of ulcer lesion as well as the ulcer
index in rats subjected to stress. Rats pretreated with kwath showed significant reduction in ulcer index at P< 0.02 while Triphala unequal showed significant reduction at P< 0.05 but reduction observed in Triphala equal group did not reach the significant level when compared with the stress control group.

The data depicted in (Fig.-9) showed a significant reduction in rectal temperature (hypothermia) in rats subjected to water immersion stress for 15 min in glass jar filled with water at 25 ± 2 °C. Rasayana or adaptogenic drugs reversed this stress - induced hypothermia. All the three Triphala formulations showed significant reduction in hypothermia almost in similar fashion in rats subjected to stress. However, the observed effect was more pronounced in Triphala unequal and kwath treated groups (P< 0.01) than Triphala equal group (P< 0.05) as compared to stress control group.

Table-18
Effect of Triphala formulations on DNA and RNA in gastric homogenate in albino rats subjected to water immersion stress

<table>
<thead>
<tr>
<th>Groups</th>
<th>DNA (µg/g tissue)</th>
<th>RNA (µg/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>641.23 ± 39.21</td>
<td>1199.24 ± 82.70</td>
</tr>
<tr>
<td>Stress control</td>
<td>228.26 ± 49.81 **</td>
<td>858.08 ± 72.37 *</td>
</tr>
<tr>
<td>Triphala equal</td>
<td>293.47 ± 60.12</td>
<td>847.74 ± 65.38</td>
</tr>
<tr>
<td>Triphala unequal</td>
<td>369.56 ± 57.51</td>
<td>951.12 ± 52.31</td>
</tr>
<tr>
<td>Chinnod. Kwath</td>
<td>369.56 ± 43.48</td>
<td>909.77 ± 54.70</td>
</tr>
<tr>
<td>Omeprazole</td>
<td>358.69 ± 53.25</td>
<td>951.12 ± 63.39</td>
</tr>
</tbody>
</table>

The values are expressed as Mean ± SEM
* P< 0.01, ** P< 0.001 compared with normal control group.

Water immersion stress significantly reduces the strength of gastric mucosa and there by reduced the resistance towards ulcers as evident by the significant decrease in the level of DNA (P<0.001) and RNA (P<0.01) in stress control group (table-18). The increased level of DNA and RNA in rats pretreated with Triphala unequal and Chinnod. kwath indicated that drugs reduced the erosion of gastric mucosa to some extent in stress condition. The observed values were comparable with the standard
<table>
<thead>
<tr>
<th>Image Description</th>
<th>Comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>Photomicrograph of stomach tissue from water immersion induced stress ulcer model</td>
<td></td>
</tr>
<tr>
<td>Stomach from normal control group (1x100) (Normal cytoarchitecture)</td>
<td>Stomach from normal control group (1x400) (Normal cytoarchitecture)</td>
</tr>
<tr>
<td>Stomach from stress control group (1x100) (Severe epithelial destruction, submucosal oedema and hemorrhage)</td>
<td>Stomach from stress control group (1x100) (Severe epithelial destruction with severe hemorrhage)</td>
</tr>
<tr>
<td>Stomach from stress control group (1x400) (Severe epithelial destruction with hemorrhage patches)</td>
<td>Stomach from Triphala equal treated group (1x100) (Submucosal oedema with hemorrhage patches)</td>
</tr>
</tbody>
</table>
Stomach from Triphala equal treated group (1x100) (Submucosal oedema with hemorrhage patches)

Stomach from Triphala unequal treated group (1x100) (Submucosal oedema)

Stomach from Triphala unequal treated group (1x100) (Mild epithelial disruption, moderate oedema in SM & ML)

Stomach from Chinnod. kwath treated group (1x100) (Moderate epithelial disruption with submucosal oedema)

Stomach from Chinnod. kwath treated group (1x100) (Mild to moderate odema in SM layer)

Stomach from omeprazole treated group (1x400) (Mild to moderate odema in SM layer)

El- Epithelial layer, SM- Submucosa, ML- Musculayer layer, SL- Serous layer
Results and observations

drug but were not of sufficient magnitude to reach statistically significant level in comparison to stress control group.

3.2. **INTESTINAL CYTOPROTECTIVE ACTIVITY**

**Evaluation of damaged small intestine by methotrexate administration**

Methotrexate was administered in dose of 12 mg/kg, orally for 4 days to albino rats. The drugs evaluated for its intestinal cytoprotective effect by studied its effect on digestive enzymes, brush border membrane enzymes, protein and lipid content, oxidants and antioxidants parameters.

**Table-19**

Effect of Triphala formulations on biochemical parameters in small intestine homogenate obtained from albino rats treated with methotrexate (MTX)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total protein (mg/g tissue)</th>
<th>Glutathione (µmoles/g tissue)</th>
<th>Total Lipid (mg/g tissue)</th>
<th>Phospholipid (mg/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>32.51 ± 2.16</td>
<td>2.36 ± 0.11</td>
<td>13.88 ± 0.84</td>
<td>3.48 ± 0.16</td>
</tr>
<tr>
<td>MTX control</td>
<td>24.64 ± 0.66 a</td>
<td>1.38 ± 0.13 c</td>
<td>10.71 ± 0.70 b</td>
<td>2.61 ± 0.14 c</td>
</tr>
<tr>
<td>Triphala equal</td>
<td>31.10 ± 2.21</td>
<td>1.89 ± 0.17 *</td>
<td>11.91 ± 0.75</td>
<td>3.14 ± 0.15 *</td>
</tr>
<tr>
<td>Triphala unequal</td>
<td>31.21 ± 2.42</td>
<td>1.94 ± 0.13 *</td>
<td>13.37 ± 0.53 *</td>
<td>3.18 ± 0.11 *</td>
</tr>
<tr>
<td>Chinnod. kwath</td>
<td>26.30 ± 2.09</td>
<td>1.38 ± 0.11</td>
<td>10.81 ± 0.63</td>
<td>2.31 ± 0.14</td>
</tr>
</tbody>
</table>

The values are expressed as Mean ± SEM

* P< 0.05 compared with MTX control group.

a P< 0.05, b P< 0.02, c P< 0.001 compared with normal control group.

Total protein, total lipid content and total glutathione levels were measured in the crude homogenate of small intestine of rats treated with methotrexate (table-19). The MTX treatment for 4 days led to significant decrease in total protein (P< 0.05), lipid content such as total lipid and phospholipid (P< 0.02 and P< 0.001) as well as cellular antioxidant such as glutathione level (P< 0.001). Triphala equal and Triphala unequal co-treatment with MTX reversed the decrease observed in total protein level but the reversal was found to be statically non-significant. Increased level of total glutathione, total lipid and phospholipid content was observed in Triphala unequal treated group ( P< 0.05) in comparison to control rats. In Triphala equal group increase was observed in the level of total glutathione and phospholipid (P< 0.05) in
comparison to MTX control group. Kwath co-treatment with MTX did not modify above mentioned biochemical changes produced by MTX in small intestine of rats.

Table-20
Effect of Triphala formulations on biochemical parameters in small intestine homogenate obtained from albino rats treated with methotrexate (MTX)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Myeloperoxidase (Unit/ g tissue)</th>
<th>Xanthine Oxidase (Unit/ g tissue)</th>
<th>Lipid peroxidation (nmoles of MDA formed/ g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>63.52 ± 2.49</td>
<td>10.27 ± 0.74</td>
<td>1.52 ± 0.13</td>
</tr>
<tr>
<td>MTX control</td>
<td>89.64 ± 2.16</td>
<td>15.35 ± 1.72</td>
<td>2.55 ± 0.18</td>
</tr>
<tr>
<td>Triphala equal</td>
<td>76.21 ± 3.81 *</td>
<td>09 55 ± 0.91 ***</td>
<td>2.25 ± 0.22</td>
</tr>
<tr>
<td>Triphala unequal</td>
<td>67.58 ± 4.61 ****</td>
<td>10.55 ± 1.03 **</td>
<td>1.82 ± 0.21 *</td>
</tr>
<tr>
<td>Chinnod. kwath</td>
<td>79.49 ± 3.99</td>
<td>09 87 ± 0.90 ***</td>
<td>2.12 ± 0.21</td>
</tr>
</tbody>
</table>

The values are expressed as Mean ± SEM
* P< 0.05, ** P< 0.02, *** P< 0.01, **** P< 0.001 compared with MTX control group.
* a P< 0.02, b P< 0.01, c P< 0.001 compared with normal control group.

Myeloperoxidase, xanthine oxidase and LPO levels were measured in the crude homogenate of small intestine of rats treated with methotrexate (table-20). Oral administration of MTX in the dose of 12 mg/kg for 4 days resulted in to significant increase in myeloperoxidase activity (P< 0.001), xanthine oxidase activity (P< 0.02) and lipid peroxidation level (P< 0.01) than that of normal control group. Myeloperoxidase (MPO) activity considered as an index of neutrophils infiltration. Significant increase in MPO activity after MTX treatment in rats suggested that this increase is due to substantial neutrophils influx in to the mucosa in response to MTX - induced injury. Triphala equal and unequal drugs significantly reduced the elevated myeloperoxidase activity (P< 0.05 and P<0.001 v/s MTX control group). The values recorded in unequal group was almost near to control group, while kwath did not induce significant modification in myeloperoxidase activity.

Lipid peroxidation and xanthine oxidase are major source of free radicals generation. The increased level of LPO was significantly attenuated by Triphala unequal at P< 0.05, while other drugs did not affect lipid peroxidation at significant level. Triphala equal and kwath reduced the xanthine oxidase activity at a significant
level of $P<0.01$ while Triphala unequal reduced the activity at the significant level of $P<0.05$.

**Table-21**

**Effect of Triphala formulations on biochemical parameters in intestinal brush border membranes vesicle (BBMV) isolated from small intestine of rats treated with methotrexate (MTX)**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total protein (mg/g tissue)</th>
<th>Na-ATPase (Unit/mg protein)</th>
<th>Ca-ATPase (Unit/mg protein)</th>
<th>Mg-ATPase (Unit/mg protein)</th>
<th>5'-Nucleotidase (Unit/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>3.89 ± 0.24</td>
<td>1.16 ± 0.059</td>
<td>0.977 ± 0.055</td>
<td>0.752 ± 0.054</td>
<td>1.90 ± 0.14</td>
</tr>
<tr>
<td>MTX control</td>
<td>2.66 ± 0.17$^b$</td>
<td>0.83 ± 0.036$^b$</td>
<td>0.740 ± 0.046$^a$</td>
<td>0.573 ± 0.034</td>
<td>1.47 ± 0.09$^a$</td>
</tr>
<tr>
<td>Triphala equal</td>
<td>3.52 ± 0.28$^*$</td>
<td>1.02 ± 0.072</td>
<td>0.965 ± 0.099</td>
<td>0.666 ± 0.102</td>
<td>1.46 ± 0.14</td>
</tr>
<tr>
<td>Triphala unequal</td>
<td>3.52 ± 0.26$^*$</td>
<td>1.05 ± 0.064$^*$</td>
<td>0.972 ± 0.053</td>
<td>0.732 ± 0.049</td>
<td>1.41 ± 0.09</td>
</tr>
<tr>
<td>Chinnod. Kwath</td>
<td>2.63 ± 0.15</td>
<td>1.05 ± 0.047$^*$</td>
<td>0.984 ± 0.054$^*$</td>
<td>0.714 ± 0.038</td>
<td>1.75 ± 0.11</td>
</tr>
</tbody>
</table>

The values are expressed as Mean ± SEM

* $P<0.05$ compared with MTX control group.

$^a$ $P<0.05$, $^b$ $P<0.01$ compared with normal control group

Table-21 depicts the data related to effects of Triphala formulations on the membrane bound ATPases and 5'-Nucleotidase and total protein content in BBMV from proximal end of small intestine treated with MTX. Methotrexate significantly reduced the level of total protein, membrane bound Na-ATPase, Ca$^{2+}$, Mg-ATPase and 5'-Nucleotidase, thus confirming that MTX can seriously damage the gastrointestinal tract. The co-administration of Triphala equal and unequal with MTX restored the protein level significantly. Na-ATPase level was significantly increased in all three treated groups when compared with the MTX control group, while the increased level of Ca$^{2+}$ and Mg-ATPase level were found to be statistically non significant. None of the drugs altered the level of 5'-Nucleotidase in BBMV of rat intestine treated with MTX.
Table-22
Effect of Triphala formulations on biochemical parameters in intestinal brush border membranes vesicle (BBMV) isolated from small intestine of rats treated with methotrexate (MTX)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Sucrase (Unit/ g protein)</th>
<th>Lactase (Unit/ g protein)</th>
<th>Alkaline PO₄ (Unit/mg protein)</th>
<th>γ-GT (Unit/ mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>220.03 ± 7.00</td>
<td>73.18 ± 3.94</td>
<td>2.53 ± 0.098</td>
<td>0.339 ± 0.014</td>
</tr>
<tr>
<td>MTX control</td>
<td>188.89 ± 2.78 ***</td>
<td>57.98 ± 3.01 **</td>
<td>2.14 ± 0.079 **</td>
<td>0.268 ± 0.075 **</td>
</tr>
<tr>
<td>Triphala equal</td>
<td>205.83 ± 8.69</td>
<td>60.52 ± 3.90</td>
<td>2.33 ± 0.080</td>
<td>0.264 ± 0 014</td>
</tr>
<tr>
<td>Triphala unequal</td>
<td>214.78 ± 5.45 *</td>
<td>70.78 ± 3.67 *</td>
<td>2.50 ± 0.080 *</td>
<td>0.303 ± 0.018</td>
</tr>
<tr>
<td>Chinnod. kwath</td>
<td>211.94 ± 4.93 *</td>
<td>66.20 ± 2.40</td>
<td>2.34 ± 0.090</td>
<td>0.315 ± 0.020</td>
</tr>
</tbody>
</table>

The values are expressed as Mean ± SEM
* P< 0.05 compared with MTX control group.
** P< 0.02, *** P< 0.01 compared with normal control group.

The level of sucrase (P< 0.01), lactase, alkaline phosphatase and γ-Glutamyl transpeptidase (P< 0.02) enzyme activities measured in the BBMV isolated from proximal end of small intestine were found to be reduced after methotrexate treatment in rats when compared with the normal control group (Table-22). Methotrexate co-treatment of rats with Triphala unequal significantly reversed the Methotrexate induced depletion of sucrase, lactase and alkaline phosphates activities (P< 0.05). But methotrexate co-treatment with Triphala equal did not affect the activity of BBMV enzymes in intestine of rats. Co-treatment with kwath enhanced the level of sucrase at P< 0.05 with no change in the level of lactase when compared with the MTX control group.
**Fig.- 10**

*Effect of Triphala formulations on phenol red permeation through the small intestine of albino rats treated with methotrexate (MTX)*

The values are expressed as Mean ± SEM

* P< 0.05 compared with MTX control group.

** P< 0.001 compared with normal control group.

The phenol red permeation through the small intestine of control rats and MTX and/or Triphala formulations treated rats were examined at 10 and 30 minutes using everted segment of small intestine as shown in (Fig.-10). The permeation through intestine in control and treated rats increased linearly between 10 to 30 min. The permeation clearance was obtained from the permeated amount of phenol red per unit length of small intestine (cm). The permeation clearance in the MTX treated rats was significantly higher than that in normal control rats. The co-administration of Triphala equal with MTX showed a slightly lower permeation clearance while co-administration of Triphala unequal with MTX showed significant decrease in permeation clearance when compared with the MTX control group.
<table>
<thead>
<tr>
<th>Photomicrograph of jejunum tissue from methotrexate induced small intestine damage model</th>
</tr>
</thead>
</table>
| **Jejunum from normal control group (1x100)**  
(Normal cytoarchitecture) |
| **Jejunum from normal control group (1x400)**  
(Normal cytoarchitecture) |
| **Colon from normal control group (1x400)**  
(Normal cytoarchitecture) |
| **Jejunum from methotrexate treated group (1x400)**  
(Marked loss of cytoarchitecture, epithelial destruction and necrosis) |
| **Jejunum from methotrexate treated group (1x400)**  
(Marked loss of cytoarchitecture, epithelial destruction and necrosis) |
| **Jejunum from Triphala equal treated group (1x100)**  
(Mild disturbance in cytoarchitecture and cell infiltration) |
Jejunum from Triphala equal treated group (1x400) (Cell infiltration)

Jejunum from Triphala unequal treated group (1x100) (Mild epithelial damage and cell infiltration)

Jejunum from Triphala unequal treated group (1x400) (Moderate cell infiltration)

Jejunum from Triphala unequal treated group (1x400) (Moderate cell infiltration)

Jejunum from Chinnod. kwath treated group (1x100) (Moderate changes to epithelial layer)

Jejunum from Chinnod. kwath treated group (1x400) (Fatty changes)

El- Epithelial layer, SM- Submucosa, ML- Musculayer layer, SL- Serous layer
3.3. COLON CYTOPROTECTIVE ACTIVITY

Induction of experimental colitis by acetic acid in albino rats.

Triphala equal, Triphala unequal and Chinnodbhavadi kwta were evaluated for its effect on serum biochemical and colon tissue biochemical parameters in experimental colitis by acetic acid in albino rats.

Table-23

Effect of Triphala formulations on serum lipid profile in acetic acid - induced experimental colitis in albino rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total Cholesterol (mg/dl)</th>
<th>HDL Cholesterol (mg/dl)</th>
<th>Triglycerides (mg/dl)</th>
<th>Phospholipid (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>65.33 ± 6.93</td>
<td>58.50 ± 8.11</td>
<td>36.50 ± 4.32</td>
<td>47.00 ± 07.30</td>
</tr>
<tr>
<td>Colitis control</td>
<td>70.33 ± 4.58</td>
<td>43.50 ± 3.17</td>
<td>93.16 ± 5.47 *</td>
<td>111.10 ± 12.74 *</td>
</tr>
<tr>
<td>Triphala equal</td>
<td>72.33 ± 9.05</td>
<td>40.50 ± 5.29</td>
<td>90.00 ± 8.39</td>
<td>101.70 ± 08.42</td>
</tr>
<tr>
<td>Triphala unequal</td>
<td>69.50 ± 3.28</td>
<td>43.00 ± 3.29</td>
<td>101.16 ± 8.77</td>
<td>94.86 ± 09.70</td>
</tr>
<tr>
<td>Chinnod. Kwath</td>
<td>62.00 ± 4.52</td>
<td>38.83 ± 2.19</td>
<td>107.50 ± 8.42</td>
<td>88.87 ± 10.73</td>
</tr>
<tr>
<td>Sulfasalazine</td>
<td>76.33 ± 2.82</td>
<td>53.50 ± 4.61</td>
<td>90.66 ± 7.77</td>
<td>82.05 ± 09.64</td>
</tr>
</tbody>
</table>

The values are expressed as Mean ± SEM
* P< 0.001 compared with normal control group.

The data related to the effect of oral administration of Triphala formulations and sulfasalazine on lipid profiles in serum of colitic rats are presented in (table-23). The acetic acid colitis resulted in to significant increase in triglycerides and phospholipid content with decrease (P>0.5) in HDL cholesterol as compared to normal control group. The test drugs did not produce any significant effect on serum lipid profiles in colitic rats compared to colitis control group.
Table-24
Effect of Triphala formulations on serum biochemical parameters in acetic acid-induced experimental colitis in albino rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Alkaline Phosphatase (IU/L)</th>
<th>Orosomucoide (mg/dl)</th>
<th>SGPT (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>70.33 ± 5.21</td>
<td>228.47 ± 21.98</td>
<td>92.53 ± 05.25</td>
</tr>
<tr>
<td>Colitis control</td>
<td>90.66 ± 5.18 **</td>
<td>337.95 ± 24.80</td>
<td>99.50 ± 18.85</td>
</tr>
<tr>
<td>Triphala equal</td>
<td>104.66 ± 4.54</td>
<td>358.59 ± 52.35</td>
<td>87.00 ± 16.95</td>
</tr>
<tr>
<td>Triphala unequal</td>
<td>92.50 ± 4.87</td>
<td>333.18 ± 42.21</td>
<td>101.83 ± 15.91</td>
</tr>
<tr>
<td>Chinnod. Kwath</td>
<td>87.16 ± 7.29</td>
<td>326.85 ± 48.02</td>
<td>97.83 ± 12.40</td>
</tr>
<tr>
<td>Sulfasalazine</td>
<td>70.66 ± 3.71 *</td>
<td>295.11 ± 51.26</td>
<td>94.16 ± 10.26</td>
</tr>
</tbody>
</table>

The values are expressed as Mean ± SEM

*P< 0.05 compared with colitis control group.

**P< 0.02 compared with normal control group.

*** P< 0.01 compared with normal control group.

The data pertaining to the effect of Triphala formulations and sulfasalazine on alkaline phosphatase, SGPT activities and orosomucoid level in acetic acid-induced colitis in rats have been presented in (table-24). Acetic acid colitis produced significant increase in the level of serum alkaline phosphatase as compared to normal control group but no change in the level of orosomucoid and SGPT could be observed. None of the drugs reversed the increased level of alkaline phosphatase to significant extent.

Table-25
Effect of Triphala formulations on serum biochemical parameters in acetic acid-induced experimental colitis in albino rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total Protein (g/dl)</th>
<th>Albumin (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>6.41 ± 0.17</td>
<td>3.65 ± 0.13</td>
</tr>
<tr>
<td>Colitis control</td>
<td>7.56 ± 0.26 ***</td>
<td>4.35 ± 0.18 ***</td>
</tr>
<tr>
<td>Triphala equal</td>
<td>6.68 ± 0.21 *</td>
<td>3.90 ± 0.13</td>
</tr>
<tr>
<td>Triphala unequal</td>
<td>6.91 ± 0.25</td>
<td>3.66 ± 0.09 **</td>
</tr>
<tr>
<td>Chinnod. Kwath</td>
<td>6.90 ± 0.30</td>
<td>3.66 ± 0.14 **</td>
</tr>
<tr>
<td>Sulfasalazine</td>
<td>6.65 ± 0.16 *</td>
<td>3.78 ± 0.18 *</td>
</tr>
</tbody>
</table>

The values are expressed as Mean ± SEM

*P< 0.05, **P< 0.02 compared with colitis control group.

*** P< 0.01 compared with normal control group.

114
As shown in (Table-25), colonic damage by acetic acid is associated with significant increase in serum total protein and albumin level in rats compared to normal control group. Triphala equal, Triphala unequal and kwath treated groups significantly reduced the elevated albumin level when compared with the colitis control group. The results were comparable with the values observed in standard group.

Table- 26

Effect of Triphala formulations on biochemical parameters in colon homogenate in albino rats with acetic acid - induced colitis

<table>
<thead>
<tr>
<th>Groups</th>
<th>Myeloperoxidase (Unit/ g tissue)</th>
<th>Lipid Peroxidation (nmoles MDA formed /g tissue)</th>
<th>Nitric Oxide (µmoles/g tissue)</th>
<th>Cathepsin D (µmol of tyrosine release /mg protein/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>52.09 ± 3.94</td>
<td>07.09 ± 0.40</td>
<td>1.028 ± 0.054</td>
<td>1.098 ± 0.017</td>
</tr>
<tr>
<td>Colitis control</td>
<td>82.87 ± 4.46 a</td>
<td>11.86 ± 0.62 b</td>
<td>1.516 ± 0.052 b</td>
<td>1.209 ± 0.100</td>
</tr>
<tr>
<td>Triphala equal</td>
<td>61.90 ± 4.18 *</td>
<td>9.40 ± 0.62 *</td>
<td>1.361 ± 0.032 *</td>
<td>1.103 ± 0.085</td>
</tr>
<tr>
<td>Triphala unequal</td>
<td>57.84 ± 3.65 **</td>
<td>9.40 ± 0.62 *</td>
<td>1.352 ± 0.036 *</td>
<td>1.187 ± 0.102</td>
</tr>
<tr>
<td>Chinnod. Kwath</td>
<td>72.73 ± 9.35</td>
<td>10.22 ± 0.96</td>
<td>1.456 ± 0.034</td>
<td>1.261 ± 0.090</td>
</tr>
<tr>
<td>Sulfasalazine</td>
<td>54.29 ± 4.97 ***</td>
<td>09.81 ± 0.47</td>
<td>1.343 ± 0.031 *</td>
<td>1.212 ± 0.048</td>
</tr>
</tbody>
</table>

The values are expressed as Mean ± SEM
*P<0.05, ** P<0.02, *** P<0.01 compared with colitis control group.
* P< 0.01, b P< 0.001 compared with normal control group

As shown in (Table-26) significant increase in mucosal myeloperoxidase activity (P<0.01), lipid peroxidation, nitric oxide expression (P<0.001) and cathepsin D activity in colonic mucosa of rats following intra-rectal administration of acetic acid compared to normal control group. Pretreatment with Triphala equal, unequal and sulfasalazine showed significant reduction in MPO activity and inducible nitric oxide synthase as compared to acetic acid control group. Lipid peroxidation is a major source of free radicals which was significantly (P<0.05) attenuated by Triphala equal and Triphala unequal drugs in colitic rats. Cathepsin D is a lysosomal enzyme and is released during disruption of the cell membrane. None of the test drugs significantly altered the level of cathepsin D in mucosa of ulcerated colon.
Results and Observations

Table-27
Effect of Triphala formulations on biochemical parameters in colon homogenate in albino rats with acetic acid - induced colitis

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total protein (mg/ g tissue)</th>
<th>LDH (µmoles of pyruvate liberated /mg protein/min)</th>
<th>Total Glutathione (nmoles/g tissue)</th>
<th>GPx (µg of GSH utilized/ mg protein/ min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>51.16 ± 1.26</td>
<td>0.598 ± 0.040</td>
<td>1668.64 ± 48.17</td>
<td>31.27 ± 0.88</td>
</tr>
<tr>
<td>Colitis control</td>
<td>57.28 ± 2.64</td>
<td>0.799 ± 0.054*</td>
<td>80889.94 ± 76.67 b</td>
<td>26.53 ± 1.12</td>
</tr>
<tr>
<td>Triphala equal</td>
<td>58.56 ± 3.04</td>
<td>0.673 ± 0.050</td>
<td>1001.19 ± 77.67</td>
<td>26.3 ± 2.24</td>
</tr>
<tr>
<td>Triphala unequal</td>
<td>52.48 ± 4.39</td>
<td>0.660 ± 0.063</td>
<td>1223.68 ± 41.25 *</td>
<td>31.55 ± 2.63</td>
</tr>
<tr>
<td>Chinnod. Kwath</td>
<td>53.76 ± 2.80</td>
<td>0.641 ± 0.023</td>
<td>1321.02 ± 78.90 **</td>
<td>26.37 ± 1.52</td>
</tr>
<tr>
<td>Sulfasalazine</td>
<td>52.16 ± 3.30</td>
<td>776.50 ± 0.061</td>
<td>1348.83 ± 128.2 **</td>
<td>27.11 ± 1.18</td>
</tr>
</tbody>
</table>

The values are expressed as Mean ± SEM

* P< 0.05, ** P< 0.01 compared with colitis control group.
* P< 0.05, b P< 0.001 compared with normal control group.

Acetic acid colitis resulted in a substantial and significant reduction of lactate dehydrogenase (LDH) (P<0.05), glutathione peroxidase (GPx) activity and glutathione (P<0.001) content in colon. This may be the result of the colonic oxidative damage induced in rats. Pretreatment with Triphala unequal, kwath and sulfasalazine to colitic animals significantly counteracted the depletion of colonic glutathione level.

It was noted that there were no significant differences observed in colonic tissue total protein level, LDH enzyme and glutathione peroxidase level in drugs treated rats compared to colitis control rats as presented in (table- 27).

Table-28
Effect of Triphala formulations on weight and length ratio and fluid absorption in colon using acetic acid - induced colitis model

<table>
<thead>
<tr>
<th>Groups</th>
<th>WL ratio (mg/cm)</th>
<th>Fluid absorption (µl/ hour. g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>095.61 ± 3.64</td>
<td>396.66 ± 11.66</td>
</tr>
<tr>
<td>Colitis control</td>
<td>135.44 ± 3.08 a</td>
<td>-74.33 ± 03.48 a</td>
</tr>
<tr>
<td>Triphala equal</td>
<td>130.93 ± 1.82</td>
<td>20.33 ± 17.66 *</td>
</tr>
<tr>
<td>Triphala unequal</td>
<td>125.80 ± 3.01</td>
<td>68.33 ± 44.75 **</td>
</tr>
<tr>
<td>Chinnod. Kwath</td>
<td>137.43 ± 4.28</td>
<td>-25.00 ± 02.88</td>
</tr>
<tr>
<td>Sulfasalazine</td>
<td>122.64 ± 2.39 *</td>
<td>143.33 ± 13.01 ***</td>
</tr>
</tbody>
</table>

The values are expressed as Mean ± SEM
Instillation of acetic acid into colon resulted in significant increase in the wet weight/length (mg/cm) ratio (P<0.001) indicating occurrence of severe oedema in vehicle treated rats compared to normal non-colitis control rats. The increased wet weight/length ratio observed in colitis control group was reduced significantly in animals pretreated with sulfasalazine treatment. However, the response obtained in groups receiving Triphala based drugs was not found be statistically significant compared to colitis control group.

In-vivo colonic fluid transport was dramatically affected by acetic acid induced colonic inflammation, changing from net absorption to net secretion as shown in (table-28). In-vivo colonic fluid absorption in vehicle treated normal rats was (396.66 ± 11.66 µl/h/g tissue). In acetic acid colitis, the net colonic fluid transport became secretory, (-74.33 ± 3.48 µl/h/g tissue). This degree of alteration in colonic fluid transport in acetic acid colitis in rats was reversed to certain extent by pre-treatment with Triphala equal (20.33 ± 17.66; P<0.05), Triphala unequal (68.33 ± 44.75; P<0.01) and sulfasalazine (143.33 ± 13.01; P<0.001) although this function value had not returned to normal function value. In Chinnodbhavadi kwath administered group, it remained on the negative side though less in comparison to colitis control group.
Fig.-11
Effect of Triphala formulations on damage score colon of albino rats using acetic acid - induced colitis model

The values are expressed as Mean ± SEM
*P< 0.05 compared with colitis control group.

Fig.-12
Effect of Triphala formulations on superoxide dismutase in colon homogenate of albino rats using acetic acid - induced colitis model

The values are expressed as Mean ± SEM
*P< 0.05 compared with colitis control group.
** P< 0.001 compared with normal control group.
The rectal administration of acetic acid induced severe macroscopic mucosal inflammation, ulceration and hemorrhagic lesions in the colon of rats, as assessed by colonic damage score. Pretreatment with Triphala unequal and sulfasalazine significantly reduced the severity of the gross lesion score. On the other hand, Triphala equal and kwath did not affect the severity of gross lesion (Fig.-11).

As shown in (Fig.-12), colonic injury following acetic acid administration was associated with profound generation of superoxide radicals or decreased activity of superoxide dismutase (SOD) (P<0.001) when compared to normal control group values. The Triphala unequal formulation significantly increased the SOD activity (P<0.05) and scavenged the O₂⁻ concentration generated in experimental ulcerative colitis. The increased level of SOD in Triphala equal and kwath treated groups was found to be statically non significant compared to colitis control group.
Photomicrograph of colon tissue from acetic acid induced ulcerative colitis model

<table>
<thead>
<tr>
<th>Colon from normal control group (1x100)</th>
<th>Colon from normal control group (1x400)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Normal cytoarchitecture)</td>
<td>(Normal cytoarchitecture)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Colon from colitic control group (1x100)</th>
<th>Colon from colitic control group (1x100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Necrosis and disruption of epithelial)</td>
<td>(Loss of cytoarchitecture, hemorrhagicspot with cell infiltration)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Colon from Triphala equal treated group (1x100)</th>
<th>Colon from Triphala equal treated group (1x100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Moderate disruption of epithelial layer)</td>
<td>(Moderate disruption of epithelial layer)</td>
</tr>
</tbody>
</table>
Colon from Chinnod. kwath treated group (1x100) (Oedema in muscular layer and mild epithelial erosion)

Colon from Triphala unequal treated group (1x100) (Mild epithelial damage)

Colon from Triphala unequal treated group (1x100) (Mild epithelial damage)

Colon from Chinnod. kwath treated group (1x100) (Mild epithelial damage)

Colon from sulfasalazine treated group (1x100) (Mild changes in epithelial layer -Cl)

El- Epithelial layer, SM- Submucosa, ML- Muscular layer, SL- Serous layer