3.1. Introduction

Gastric hypersecretion is a classic and well-established threat for peptic ulcer production (Levenstein, 1999). Despite the multifactorial etiology of peptic ulcer the suppression of acid secretion is main approach for preventing peptic ulcer disease, related symptoms and complications (Golbabapour et al, 2013; Katzung et al, 2012). The pyloric ligation model was therefore used for observing the acid suppression aspect of Triphala. Irrespective of etiology the mucosal derangement, higher acid secretion and free radicals production are involved in pathogenesis of peptic ulcers (Gulia and Chaudhary, 2011, Das 1997). Therefore parameters like mucus thickness, total acidity and free radicals scavenging enzymes (antioxidant enzymes) such as MDA (TBARS), Glutathione, Catalase and SOD (superoxide dismutase) were evaluated in this study. These are well established scientific parameters for the evaluation of gastro-protective activity of any drug (Parmar and Desai, 1993; Adinortey et al, 2013).

3.2. Materials and Methods

3.2.1. Materials: Same as in chapter 2

3.2.2. Methods:

3.2.2.1. Preparation of Carboxymethyl cellulose (CMC) vehicle: Same as in chapter 2

3.2.2.2. Preparation of Indomethacin suspension: Same as in chapter 2

3.2.2.3. Preparation of Ranitidine suspension: Same as in chapter 2

3.2.2.4. Preparation of Triphala extract: Same as in chapter 2

3.2.2.5. Dose selection: Same as in chapter 2

3.2.2.6. Selection of animals: Same as in chapter 2
3.2.2.7. Experimental Framework

In Indomethacin + Pyloric ligation model only the preventive approach was adopted to evaluate the antisecretory efficacy of Triphala. As in curative approach of treatment animals should be survived after disease induction which would be very difficult after abdominal stitching and pyloric ligation.

In preventive model approach the animals were pretreated with the test compounds and their protective effect was observed through inducing the disease (gastric ulcer). Before inducing the disease animals were kept on fasting for 24-36 hours allowing free access to drinking water. All animals were kept in wire mesh cage during fasting to avoid coprophagy.

3.2.2.7.1. Indomethacin (20mg/kg, oral) + pylorus ligation induced gastric ulcer
(Parmar and Desai, 1993).

Treatment protocol: It is year old rat model of gastric ulcers invented by Shay et al (1945). In this model the antiulcer activity of Triphala preparation was investigated using five groups of Wistar Albino rats (n= 5) for the prescribed duration. The first (normal control), second (pylorus control) and third (ulcer control) group animals received 1% CMC (1mg/kg, oral) for 21 days. The fourth group i.e. drug treated group was administered 70% ethanolic extract of Triphala preparation at the dose of 1000 mg/kg, oral for 21 days, Ranitidine (50 mg/kg, oral) was administered for 21 days to the fifth group (standard group). After 21 days of dosing, the animals of group III, IV and V were given Indomethacin (20 mg/kg, oral) before 6 hours of pyloric ligation with 24 hours fasting.
Table 3.1: Treatment schedule in preventive model of Indomethacin (20mg/kg, oral) + Pylorus ligation induced gastric ulcer

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. of animals</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>6</td>
<td><strong>Vehicle control (Group I)</strong>&lt;br&gt;Pretreatment with CMC [1ml (1%)/kg/day, oral] for 21 days, animals was sacrificed on last day of treatment after 24hrs fasting</td>
</tr>
<tr>
<td>II</td>
<td>6</td>
<td><strong>Pylorus control (Group II)</strong>&lt;br&gt;Pretreatment with CMC [1ml (1%)/kg/day, oral] for 21 days + pylorus ligation on 21st day under ketamine anaesthesia on 24 hrs fasted rats</td>
</tr>
<tr>
<td>III</td>
<td>6</td>
<td><strong>Ulcer control (Group III)</strong>&lt;br&gt;Pretreatment with CMC [1ml (1%)/kg/day, oral] for 21 days + ulcer induction by Indomethacin administration (20mg/kg, oral) on 24 hrs fasted rats + pylorus ligation after 6 hr of Indomethacin administration.</td>
</tr>
<tr>
<td>IV</td>
<td>6</td>
<td><strong>Triphala treated group (Group IV)</strong>&lt;br&gt;Pretreatment with Triphala [1g/kg/day, oral] for 21 days + ulcer induction by Indomethacin administration (20mg/kg, oral) on 24 hrs fasted rats + pylorus ligation after 6 hrs of Indomethacin administration.</td>
</tr>
<tr>
<td>V</td>
<td>6</td>
<td><strong>Standard drug treated group (Group V)</strong>&lt;br&gt;Pretreatment with Ranitidine (50 mg/kg, oral) for 21 days + ulcer induction by Indomethacin administration (50mg/kg, oral) on 24 hrs fasted rats + pylorus ligation after 6 hrs of Indomethacin administration.</td>
</tr>
</tbody>
</table>
After treating animals as mentioned in above table 3.1 pylorus ligation was done in each rat under ketamine (45 mg/kg, i.p.) anesthesia (Chandrashekhar et al, 2010). After anaesthising the rat abdomen was opened by a small midline incision below the xiphoid process, pyloric portion of the stomach was slightly lifted out and ligated avoiding traction to the pylorus or damage to its blood supply. The stomach was replaced carefully and the abdominal wall closed by interrupted sutures. The animals were deprived of both food and water during the postoperative period and gastric juice was allowed to accumulate for a period of four hours. The rats were then sacrificed by cervical dislocation and stomach was removed after clamping their oesophagus. The gastric contents were then collected through the oesophagus into test tube. They were centrifuged at 3000 rpm for 20 minutes. The supernatant was subjected to analysis for titrable acidity and total volume of gastric juice (Parmar and Desai, 1993). The stomach was exposed along the side of greater curvature, cleaned with distilled water carefully and evaluated for lesions (Vogel, 2002). After evaluation, a portion of stomachs was weighed and instantaneously dipped in solution of alcian blue for mucus wall thickness determination (Corne et al, 1974). After that a small portion of stomach was further taken and kept in 10% of formalin solution for histological studies. A small portion was further taken, weighed and homogenized in 0.02 M EDTA for estimation of Glutathione and remaining stomach was homogenised after weighing in buffer of sodium phosphate (50 mM, pH 7.4) for study of antioxidant parameters such as TBARS (thiobarbituric acid reactive substances), catalase and SOD (superoxide dismutase). The homogenised buffer solutions and stomach portions for mucus barrier determination were kept in deep freezer at -20 ºC until further analysis.
3.2.2.8. Tissue processing: Same as in chapter 2

3.2.2.9. Homogenization: Same as in chapter 2

3.2.2.10. Subcellular fractionation: Same as in chapter 2

3.2.2.11. Parameters Assessed

1. Ulcer index (Dashputre and Naikwade, 2011): Same as in chapter 2

2. Gastric wall mucus (in gastric tissue) (Corne et al, 1974): Same as in chapter 2

3. Total acidity (in gastric juice) (Parmar et al, 1986)

   For the measurement of total acidity of stomach juice of the samples, a measured volume of the stomach juice was titrated with 0.01N sodium hydroxide to pH 8.5 with phenolphthalein as indicator (Parmar et al. 1986). The unit of total acidity was expressed as mill equivalents per liter per 4 hours (meqv/l/4h). The reading of total acidity of the test samples of stomach juice with a pH more than 2.5 or mixed with blood were not incorporated in the data.

4. Volume of gastric juice (Vogel, 2002)

   The rats were sacrificed by cervical dislocation and stomachs were removed after clamping their oesophagus. The stomach contents were then collected through the oesophagus for measurement of volume. After centrifugation at 3000 rpm for 20 minutes the supernatant was evaluated for examination of titrable acidity and total volume of gastric juice (Parmar and Desai, 1993).

5. In vivo antioxidant activity (in gastric tissue)
   I. Estimation of Thio Barbituric Acid Reactive Substances (TBARS) (Ohkawa et al, 1979)
   II. Estimation of glutathione (Sedlak and Lindsay, 1968)
   III. Estimation of Catalase (CAT) (Clairbone et al, 1985)
   IV. Estimation of Super oxide dismutase (SOD) (Marklund et al, 1974)
3.3. Result

3.3.1. Preventive effect of Triphala in Indomethacin + pylorus ligation induced gastric ulcer

The present study showed that vehicle control group (i.e. group I), which was fed only with the basal diet along with CMC produced control levels of ulcer index, gastric wall mucus thickness and antioxidant parameters such as TBARS, catalase, glutathione and SOD and animals in CMC + pylorus ligated group (i.e. group II rats) showed control levels of total acidity and volume of gastric juice.

Effect on ulcer index (Table 3.2, Figure 3.1): -

The group II (i.e. CMC+ Pylorus ligation) and group III animals (i.e. Indomethacin + Pylorus ligation group) showed significant increase in the mean ulcer index (P < 0.01) as compared to group I rats i.e. normal control healthy rats or vehicle control group.

The significant increase in mean ulcer index was also observed in group III animals (i.e. Indomethacin + Pylorus ligation group) as compared to group II ((i.e. CMC+ Pylorus ligation).

While the group IV i.e. Triphala treated (1.0 g /kg/oral) and Ranitidine (standard drug treated rats,50 mg/kg/oral) i.e. group V showed significant decrease in the mean ulcer index (P < 0.01) as compared to the group III animals (i.e. Indomethacin + Pylorus ligation group).

No significant (p>0.05) decrease in mean ulcer index was found in Triphala treated group (i.e. Group IV rats) as compared to the mean ulcer index in standard drug treated rats (i.e. Group V).
Effect on gastric wall mucus (in gastric tissue) (Table 3.2, Figure 3.2):- The group II (CMC+ Pylorus ligation) and group III (i.e. Indomethacin + Pylorus ligation group) showed significant decrease (P<0.01) in the mean gastric wall mucus thickness as compared to normal control group I (i.e. vehicle control group).

On comparison with control group II (CMC+ Pylorus ligation) rats significant decrease (P<0.05) in mean gastric wall mucus thickness was observed in group III rats (i.e. Indomethacin + Pylorus ligation group).

The group IV rats i.e. Triphala treated rats at the dose of 1.0 g/kg/oral and group IV rats i.e. Ranitidine treated at the dose of 50 mg/kg/oral showed significantly high (P<0.01) gastric mucus content as compared to group III animals (i.e. Indomethacin + Pylorus ligation group).

Whereas, no significant (p>0.05) increase in mean gastric wall mucus thickness was shown in Triphala treated group (i.e. Group IV rats) as compared to the mean gastric wall mucus thickness in standard drug treated rats (i.e. Group V).

Effect on total acidity of gastric juice (Table 3.2, Figure 3.3): -

The mean total acidity of gastric juice was significantly (p<0.01) increased in Indomethacin treated pylorus ligated rats (i.e. group III rats) as compared to mean total acidity of gastric juice in CMC + pylorus ligation treated rats (i.e. group II). Triphala treatment in the dose of 1.0g/kg/oral (i.e. Group IV rats) significantly (p<0.01) decreased mean total acidity as compared to Indomethacin treated pylorus ligated rats (i.e. Group III).

Further, in the standard drug treated rats (Ranitidine 50mg/kg/oral i.e. Group V), the mean total acidity was significantly (p<0.01) decreased as compared to the mean total acidity in the Indomethacin treated pylorus ligated rats (i.e. Group III rats).
However, no significant (p>0.05) decrease in mean total acidity was shown in Triphala treated group (i.e. Group IV rats) as compared to the mean total acidity in standard drug treated rats (i.e. Group V).

**Effect on volume of gastric juice (Table 3.2, Figure 3.4):**

The significant (p<0.01) increase in mean volume of gastric juice was found in Indomethacin treated pylorus ligated rats (i.e. Group III) as compared to mean volume of gastric juice in CMC + pylorus ligation rats (i.e. Group II rats).

While Triphala treatment in the dose of 1g / kg/ oral (i.e. Group IV rats) caused decrease in mean gastric juice volume significantly (p<0.01) as compared to Indomethacin treated pylorus ligated rats (i.e. Group III rats).

The mean volume of gastric juice was decreased significantly (p<0.01) in standard drug treated rats also (Ranitidine 50mg/kg /oral i.e. Group V) as compared to Indomethacin treated pylorus ligated rats (i.e. Group III).

However, no significant (p>0.05) difference in mean gastric juice volume was observed in Triphala treated group (i.e. Group IV rats) as compared to the mean gastric juice volume in standard drug treated rats (i.e. Group V).

**Effect on TBARS (in gastric tissue) (Table 3.3, Figure 3.5):** The CMC+ Pylorus ligation treated rats (i.e. Group II) and CMC+ Indomethcin treated rats (i.e. Group III) showed significant increase in TBARS level (P < 0.05 and P < 0.01 respectively) as compared to control group I.

Significant increase (P<0.05) in TBARS level was observed in group III rats (i.e. Indomethacin + Pylorus ligation group) as compared to control group II (CMC+ Pylorus ligation).
The group IV (Triphala, 1g/kg/oral) and V (Ranitidine, 50 mg/kg/oral) showed significant decrease in the TBARS levels (P< 0.01) as compared to group III (i.e. Indomethacin + Pylorus ligation group).

Furthermore, the mean TBARS level was not significantly (p>0.05) decreased in Triphala treated group (i.e. Group IV rats) as compared to the mean TBARS level in standard drug treated rats (i.e. Group V).

**Effect on Glutathione level (in gastric tissue) (Table 3.3, Figure 3.6):**-Significant low level of the glutathione (P<0.01) was observed in group II (CMC+ Pylorus ligation) and group III (i.e. Indomethacin + Pylorus ligation group) as compared to normal control healthy rats (i.e. group I rats).

Further no significant change (P>0.05) in glutathione level was observed in group III rats (i.e. Indomethacin + Pylorus ligation group) as compared to control group II (CMC+ Pylorus ligation).

While in group IV i.e. Triphala treated (P< 0.05) and in group V i.e. Ranitidine treated rats (P< 0.01) the glutathione level was significantly increased as compared to group III rats (i.e. Indomethacin + Pylorus ligation group).

Whereas in Triphala treated group (i.e. Group III rats) the mean glutathione level was not significantly (p>0.05) changed as compared to the mean glutathione level in standard drug treated rats (i.e. Group IV).

**Effect on catalase(in gastric tissue) (Table 3.3, Figure 3.7):**-It was observed that the catalase level was decreased significantly (P< 0.01) in group II (i.e. CMC+ pyloric ligation rats) and group III (i.e. Indomethacin + Pylorus ligation group) as compared to mean catalase level of group I (i.e. normal control group).
However no Significant change (P>0.05) in catalase level was seen in group III rats (i.e. Indomethacin + Pylorus ligation group) as compared to control group II (CMC+ Pylorus ligation).

Triphala treated rats (i.e. Group IV) at the dose of 1g/kg/oral showed that catalase level was significantly increased as compared with group III (i.e. Indomethacin + Pylorus ligation group).

In standard drug treated rats (Ranitidine 50mg/kg/day) significantly (P<0.01) increased catalase level was observed as compared with group III.

Furthermore the non significant (p>0.05) decrease in mean catalase level was observed in Triphala treated group (i.e. Group III rats) as compared to mean catalase level of standard drug treated rats (Ranitidine 50 mg/kg/oral (i.e. Group IV).

**Effect on SOD (in gastric tissue)** (Table 3.3, Figure 3.8):- Superoxide dismutase level (P< 0.01) was decreased in CMC+ Pyloric ligation group (i.e. Group II) as compared to vehicle control group (i.e. Group I).

In Indomethacin + Pylorus ligation group (i.e. Group III) significantly decreased (P<0.01) SOD level was also observed as compared to normal control healthy rats (i.e. Group I).

SOD level, however did not significantly change (P>0.05) in group III rats (i.e. Indomethacin + Pylorus ligation group) as compared to control group II (CMC+ Pylorus ligation).

The SOD level in Triphala treated rats (i.e. Group IV rats) and standard drug treated rats (i.e. Group V rats) showed significant increase (P<0.01) in SOD level as compared with Indomethacin + pyloric ligation group (i.e. Group III).
Whereas not significant (p>0.05) decrease in mean SOD level was seen in Triphala treated group (i.e. Group IV rats) as compared to mean SOD level of standard drug treated rats (i.e. Group V).

**Macroscopical evaluation:** Description of macroscopical evaluation is mentioned in Figure 3.9

**Histological evaluation:** Description of histological evaluation is mentioned in Figure 3.10 (A) (B).
Table 3.2: Effect of Triphala on ulcer index and mucus on Indomethacin and pyloric ligation induced gastric ulcer

<table>
<thead>
<tr>
<th>Groups</th>
<th>Ulcer score</th>
<th>Mucus (µg/g of stomach tissue)</th>
<th>Total acidity (meq/lit)</th>
<th>Vol. of gastric juice (ml/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (Vehicle control)</td>
<td>0.00</td>
<td>206.35±8.7</td>
<td>___</td>
<td></td>
</tr>
<tr>
<td>Pretreatment with CMC [1ml (1%)/kg/day, oral] for 21 days, animals were sacrificed on last day of treatment after 24hrs fasting</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group II (Pylorus control)</td>
<td>15.5 ± 0.5**</td>
<td>139.58±7.2**</td>
<td>51.15±0.82</td>
<td>3.84 ±0.13</td>
</tr>
<tr>
<td>Pretreatment with CMC [1ml (1%)/kg/day, oral] for 21 days + pylorus ligation on 21\textsuperscript{st} day under ketamine anaesthesia on 24 hrs fasted rats</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group III (Ulcer control)</td>
<td>37.25±1.75**,@@</td>
<td>91.71±4**,@@</td>
<td>81.30 ± 0.65@@</td>
<td>2.43±0.16@@</td>
</tr>
<tr>
<td>Pretreatment with CMC [1ml (1%)/kg/day, oral] for 21 days + ulcer induction by Indomethacin administration (20mg/kg, oral) on 24 hrs fasted rats + pylorus ligation after 6 hr of Indomethacin administration</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group IV (Triphala treated group)</td>
<td>22.3 ± 0.98##,ns</td>
<td>185.8±7.4##,ns</td>
<td>41.00±0.49##,ns</td>
<td>3.26±0.21##,ns</td>
</tr>
<tr>
<td>Pretreatment with Triphala [1ml (1%)/kg/day, oral] for 21 days + ulcer induction by Indomethacin administration (20mg/kg, oral) on 24 hrs fasted rats + pylorus ligation after 6 hrs of Indomethacin administration</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group V (Standard drug treated)</td>
<td>25.1±1.5##</td>
<td>180.5±18##</td>
<td>42.07±0.49##</td>
<td>3.56±0.13##</td>
</tr>
<tr>
<td>Pretreatment with Ranitidine (50mg/kg/day) for 21 days+ ulcer induction by Indomethacin administration (20mg/kg, oral) on 24 hrs fasted rats + pylorus ligation after 6 hrs of Indomethacin administration</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All values are expressed as Mean ± SEM (n=5), ** P<0.01, as compared to vehicle control group (i.e. Group I), @ P<0.05, @@ P<0.01, as compared to pyloric control group (i.e. Group II),## P<0.01, as compared to Indomethacin + pyloric control group (i.e. Group III), ns P>0.05, as compared to standard drug treated group (i.e. Group V)
Figure 3.1-Preventive effect of Triphala on ulcer index in indomethcin+pyloric ligation induced gastric ulcers

![Ulcer Index Score Chart]

Figure 3.2-Preventive effect of Triphala on mucus in indomethcin+pyloric ligation induced gastric ulcers

![Gastric Wall Mucus Chart]

Figure 3.1 and 3.2-All values are expressed as Mean ± SEM (n=5), ** P<0.01, as compared to vehicle control group (i.e. Group I), @ P<0.05, @@ P<0.01, as compared to pyloric control group (i.e Group II),## P<0.01, as compared to ulcer control (Indomethacin + pyloric) group (i.e Group III),ns P>0.05, as compared to standard drug treated group (i.e. Group V)
Figure 3.3-Preventive effect of Triphala on total acidity in indomethcin + pyloric ligation induced gastric ulcers

Figure 3.4-Preventive effect of Triphala on volume of gastric juice in indomethcin+pyloric ligation induced gastric ulcers

Figure 3.3 and 3.4-All values are expressed as Mean ± SEM (n=5), @@ P<0.01, as compared to pyloric control group (i.e Group II),## P<0.01, as compared to ulcer control (Indomethacin + pyloric) group (i.e Group III), ns P>0.05, as compared to standard drug treated group (i.e. Group V)
Table 3.3: Preventive effect of Triphala on TBARS, Glutathione, Catalase, Superoxide dismutase in Indomethacin + pyloric ligation induced gastric ulcer.

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>TBARS</th>
<th>GLUTATHIONE</th>
<th>CATALASE</th>
<th>SUPEROXIDE DISMUTASE</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (Vehicle control)</td>
<td>0.063 ± 0.001</td>
<td>18.81 ± 0.59</td>
<td>0.157 ± 0.01</td>
<td>0.588 ± 0.02</td>
</tr>
<tr>
<td>II (Normal pyloric control)</td>
<td>0.122 ± 0.012*</td>
<td>7.624 ± 0.728**</td>
<td>0.062 ± 0.011**</td>
<td>0.365 ± 0.049**</td>
</tr>
<tr>
<td>III (Indomethacin+pyloric ligation)</td>
<td>0.150±0.014**,*@</td>
<td>6.364 ± 0.694**,*5</td>
<td>0.051±0.011**,*5</td>
<td>0.333±0.041**,*5</td>
</tr>
<tr>
<td>IV (Drug treated group)</td>
<td>0.072 ± 0.008##,ns</td>
<td>10.41 ± 1.295##,*ns</td>
<td>0.128 ± 0.013##,ns</td>
<td>0.554 ± 0.079##,*ns</td>
</tr>
<tr>
<td>V (Standard drug treated group)</td>
<td>0.082 ± 0.008##</td>
<td>11.33 ± 1.023##</td>
<td>0.132 ± 0.013##</td>
<td>0.588 ± 0.055##</td>
</tr>
</tbody>
</table>

All values are expressed as Mean ± SEM (n=5), * P<0.05, ** P<0.01, as compared to vehicle control group (i.e. Group I), @ P<0.05, $ P>0.05, as compared to pyloric control group (i.e Group II), # P<0.05, ## P<0.01, as compared to Indomethacin + pyloric group (i.e. Group III), ns P>0.05, as compared to standard drug treated group (i.e. Group V)
Figure 3.5 - Preventive effect of Triphala on TBARS in indomethacin+pyloric ligation induced gastric ulcers

Figure 3.6 - Preventive effect of Triphala on Glutathione in indomethacin+pyloric ligation induced gastric ulcers

Figure 3.5 and 3.6 - All values are expressed as Mean ± SEM (n=5), * P<0.05, ** P<0.01, as compared to vehicle control group (i.e. Group I), @ P<0.05, $ P>0.05, as compared to pyloric control group (i.e Group II), # P<0.05, ## P<0.01, as compared to ulcer control (Indomethacin + pyloric) group (i.e. Group III), ns P>0.05, as compared to standard drug treated group (i.e. Group V)
Figure 3.7-Preventive effect of Triphala on Catalase in indomethcin+pyloric ligation induced gastric ulcers

Figure 3.8-Preventive effect of Triphala on Superoxide dismutase in indomethcin+pyloric ligation induced gastric ulcers

Figure 3.7 and 3.8-All values are expressed as Mean ± SEM (n=5), ** P<0.01, as compared to vehicle control group (i.e. Group I), $ P>0.05$, as compared to pyloric control group (i.e Group II), ## P<0.01, as compared to ulcer control (Indomethacin + pyloric) group (i.e. Group III), ns P>0.05, as compared to standard drug treated group (i.e. Group V)
Figure 3.9- Preventive effects of Triphala extract on macroscopic appearance of the gastric mucosa in Indomethacin+pyloric ligation induced gastric ulcers

(a) The normal control group shows intact gastric mucosa tissue (b) Moderate lesions are seen in CMC+pyloric ligation group (c) Severe lesions are observed in the gastric mucosa of the ulcer control group. Indomethacin+pyloric ligation produced highly visible hemorrhagic necrosis of the gastric mucosa. (d) In the experimental groups, rats pretreated with 1000 mg/kg, oral of the Triphala extract shows mild lesion in the gastric mucosa (e) The standard drug treated group, pretreated with Ranitidine (50mg/kg), also shows mild lesions to the gastric mucosa.
Figure 3.10 (A) - Histological section of gastric mucosa in indomethacin+pyloric ligation induced gastric ulcers (preventive model) under 10x microscope (H&E staining)
Figure 3.10(B) - Histological section of gastric mucosa in indomethacin+pyloric ligation induced gastric ulcers (preventive model) under 40x microscope (H&E staining)

A) Group I (normal control) showing normal architecture of gastric tissue. (B) Group II (CMC+pyloric ligation) control with mild disruption of surface epithelium and little oedema of submucosal layer (C) Group III (Indomethacin+pyloric ligation) ulcer control showing extensively damaged and disorganized gastric tissue, significantly high disruption to the surface epithelium and oedematous submucosal layer. (D) & (E) Group IV (Triphala treated) and Group V (standard drug treated) showing significantly better protection of the gastric mucosa verified by significant reduction or absence of the ulcer area, submucosal oedema and epithelial cell disorganization.
3.4. Discussion

Oversecretion of gastric acid is a distinctive and well-established warning for peptic ulcer creation (Levenstein 1999). Despite the multifactorial origin of peptic ulcer the repression of acid secretion is main approach for preventing peptic ulcer disease, related symptoms and complications (Golbabapour et al, 2013; Katzung et al, 2012).

It is evident that gastric acid discharge aggravates all types of peptic ulcers irrespective of etiology either basal acid secretion is normal or low. Moreover, in all types of peptic ulcer suppression of intragastric acidity is main approach of treatment either by diminishing the secretion of gastric acid or by neutralizing the already secreted acid in stomach. Many conventional drugs such as anticholinergic (pirenzepine, propantheline, oxyphenonium), H₂ antihistamines (Ranitidine, roxatidine, famotidine, cimetidine) inhibitors proton pump (PPI) (omeprazole, lansoprazole, pantoprazole, rabeprazole, esomeprazole), prostaglandin analogue (misoprostol) reduce the secretion of gastric acid whereas oral antacids {sodium bicarbonate, sodium citrate, magnesium hydroxide, magnesium trisilicate, aluminium hydroxide, magaldrate (hydrated complex of hydroxyl magnesium aluminate), calcium carbonate} neutralize the secreted gastric acid (Katzung et al, 2012; Tripathi 2014; Rang et al, 2014).

As acid release suppression is main approach of treatment therefore the pyloric ligation model was used for observing the acid suppressive aspect of Triphala. In this type of model the stomach juice is allowed to accumulate for particular time in stomach. The pylorus end of stomach is ligated by suture by a simple surgical procedure.

The basis of creation of gastric ulcers by pyloric ligation are supposed to because of stress induced augment in stomach hydrochloric acid release, stasis of secreted acid and the volume of secretion. These are significant factors in the ulcer formation by accumulated stomach juice which is in contact with unguarded mucosa of the stomach. Auto digestion
of the stomach mucosa and breakdown of the gastric mucosal fence are responsible for ulcer induction in Pylorus ligation model (Dashputre and Naikwade 2011). Obstruction of gastric blood flow (Patel et al., 2000) and production of free radicals is also accountable for the formation of ulcer (Rastogi et al., 1998, Zaghlool et al, 2015). Perhaps basement membrane is disrupted by gastric acid to make profound injury (Chan and Leung, 2002).

The vehicle control group (i.e. group I) in the present study which was fed only with the basal diet along with CMC produced normal levels of ulcer index score, gastric wall mucosal thickness, and antioxidant enzymes (TBARS, Glutathione, Catalase and SOD) and animals in CMC + pylorus ligation group (i.e. group II rats) showed normal levels of total acid and gastric juice volume.

It was observed that Indomethacin (20 mg/kg/oral) treated rats of pylorus ligated group (i.e. group III rats) in the present study resulted in significant increase in mean ulcer index, TBARS and significant alleviation in mean gastric wall mucus thickness, catalase, glutathione and SOD with respect to the healthy rats of normal control group (i.e. group I). Further, the mean total acidity of stomach juice was significantly elevated and there was significant alleviation in volume of stomach juice happened in Indomethacin (20 mg/kg/oral) treated rats of pyloric ligated group (i.e. group III rats) with respect to CMC + pylorus ligated rats (i.e. group II rats).

The consequence of test drug, viz. Triphala in the dose of 1.0 g/kg/oral i.e., group IV and the rats of group V treated with standard drug (Ranitidine, 50 mg/kg/oral) were compared with that of rats of Indomethacin treated pyloric ligation group (i.e. group III rats).

Various scoring methods have been developed which quantitate gastric ulceration by calculating ulcer indices. Though in all the scoring systems, the number of ulcers has
been given the utmost importance in the assessment of severity, other parameters as the ratio between the total gastric mucosal area and the area of ulceration and/or perforation and the frequency distribution of ulcer size should not be overlooked. Extent of score of ulcer index is considered as the marker of gastric mucosal damage by ulcerogenic agents it may be stress, drug or any other factor (Parmar and Desai, 1993).

A significant decline was seen in mean ulcer index in Triphala pre-treated rats (i.e., group IV) and the standard drug (Ranitidine) pre-treated rats (i.e. group V rats) as compared to the rats of indomethacin treated pylorus ligated group (i.e. group III rats). However no significant (p>0.05) change was seen in ulcer index in Triphala pre-treated group on comparison with standard drug treated group.

The decline in ulcer index is in conformity with those of Oloyede et al, (2015) who reported the decrease in ulcer index on treatment with carica papaya seed in Indomethacin treated pylorus ligated rats. Decline in ulcer index was also described by Agrawal et al, (2000) with treatment by Zingiber officianalis Linn, Piper longum Linn and species of ferula in rats of pylorus ligation model.

The contribution of mucosal barrier in formation of peptic ulcer has been looked very attentively and the word “cytoprotection” coined for it i.e., defense of mucosal damage by other means besides the diminishing of acid secretion (Robert, 1981). Now it is well-known that the disease of peptic ulcer can be treated by reinforcing the defensive aspects of stomach and duodenum mucosa rather than suppressing aggressive factors (Dhuley, 1999).

In the present study, pre-treatment with Triphala at the dose level of 1.0 g /kg/oral (group IV) and the standard drug (Ranitidine 50 mg/kg/oral, i.e. group V) causes significant increase in the mean thickness of gastric wall mucus on comparison with the
Indomethacin treated rats of pylorus ligated group (i.e. group III rats). Increment in mucus layer thickness with Triphala matches the results of Dhuley (1999) where he depicted an rise in mucus thickness of gastric wall with rhinax (a herbal formulation consists of water extracts of medicinal plants, namely Withania somnifera L. (root), Asparagus racemosus Willd.(root), Mucuna prurience(root), Emblica officinalis Gaertn.(fruit), Myristica fragrance Houtt.(seed), Glycyrrhiza glabra L. (root) ) treated rats against chemical and physical factors-induced peptic ulcers. Whereas pre-treatment with Triphala presented no significant change in thickness of gastric wall mucus on comparison with standard drug treated group.

Association of excessive acid production with peptic ulcers has been proven by many researchers (Brodie et al, 1962, Levine and Senay 1970, Dai and Ogle 1975).

The belief in term “no acid-no ulcer” and the remedies used to lessen acid release has ruled in drug treatment of peptic ulcer for many decades (Freston, 1990).

The significant increase in the mean total acidity was exhibited in Indomethacin treated pylorus ligated rats (i.e. group III rats) when compared to the mean total acidity of rats of CMC administered pylorus ligated group (i.e. group II rats).

The Triphala pre-treated rats in dose of 1.0g/kg/oral for a period of 21 days when challenged with Indomethacin+pylorus ligation, it was observed that there has been significant (p<0.01) decrease in the mean total acidity in Triphala pre-treated rats (i.e. group IV) as compared to Indomethacin treated pylorus ligated rats (i.e. group III rats).

When standard drug, Ranitidine (50 mg/kg /oral) i.e. group V rats were compared with Indomethacin treated pylorus ligated rats (i.e. group III rats), then, there was a decrease in mean total acidity and the decrease was significant (p<0.01). But, when Triphala pre-
treated group was compared with respect to standard drug Ranitidine treated group no significant reduction was seen in mean total acidity.

These results substantiate the finding of Surender (1999), where he observed a decline in mean total acidity in aspirin challenged pylorus ligated rats with pre-treatment of fixed oil of *Ocimum basilicum* Linn.

Studies have been proven that reduction of gastric secretion safeguards the gastroduodenal mucosa from the insults caused by pylorus ligation (Parmar *et al*, 1986). Sanmugapriya and Venkataraman (2007) reported that decline in volume of stomach juice reduce the total acidity and so ulcer index.

Significant elevation was occurred in mean stomach juice volume in Indomethacin treated pylorus ligated rats (i.e. group III rats) with respect to the mean volume of stomach juice in CMC treated pylorus ligated rats (i.e. group II rats).

Significant (p<0.01) diminution was occured in mean volume of stomach juice with Triphala, given in the dose of 1 g/kg/oral (i.e., group IV rats) as well as in standard drug Ranitidine (50 mg/kg/oral) i.e. group V rats when compared to the Indomethacin treated rats of pylorus ligated group (i.e. group III rats). However, this diminution in mean volume of stomach juice was not significant (p>0.05) in Triphala pre-treated group on comparison with standard drug pre-treated group.

However pre-treatment with Triphala did not show any significant (p>0.05) decline in mean volume of stomach juice on comparison with standard drug pre-treated group.

This study confirm the result of Surender (1999), Sanmugapriya and Venkataraman (2007) where they evaluated the antisecretoy action of *Ocimum basilicum* Linn. and *Strychnous potatorum* Linn. seeds respectively.
Generation of free radicals of oxygen and peroxidation of lipid plays a key function in the progress of the gastric mucosal lesions caused by Indomethacin plus pyloric ligation (Rastogi et al., 1998, Zaghlool et al, 2015). These are indicated by results of the report that the intensity of lipid peroxide or MDA was increased in Indomethacin treated pylorus ligated rats (i.e. group III rats) as compared to healthy rats of normal control (group I). In contrast, pre treatment of the rats with Triphala extract and standard drug Ranitidine attenuated the level of lipid peroxide or MDA(TBARS) as compared to that Indomethacin treated pylorus ligated rats (i.e. group III rats). However this attenuation in Triphala pre-treated group is not significant as compared to standard drug treated group. Thus, Triphala extract pre-treatment has reduced the lipid peroxidation process in rats.

The level of marker of free radical scavenging enzymes such as GSH, CAT and SOD significantly decreased in Indomethacin treated pylorus ligated rats (i.e. group III rats) as compared to (group I) rats i.e normal control healthy rats. However, Pre-treatment of the rats with Triphala extract as well as standard drug (Ranitidine) restored the depleted level of these enzymes. Further in Triphala pre-treated group this depletion was not significant as compared to standard drug Ranitidine treated group. These results suggested the possible involvement of endogenous anti-oxidants in the experimental protective effect of Triphala extract in gastric ulcer.

Repair of stomach mucosa is both by restitution and cell proliferation. In recent times, a number of polyphenolics have been originated to have a protective action on gastric damage in rats.

A number of researchers have paid attention on the antiulcer effect of polyphenol. This action was mainly described by strong antioxidant potential and/or by many other factors, such as powerful protein-binding capability, modification of leukocyte gathering and mucus production and restitution (Shakeerabanu et al, 2011). Triphala is enriched with
tannins and mucus membrane integrity is affected by it. Tannins beneficial effect in preventing ulcer development could be due to their vasoconstriction and protein precipitating properties (Nariya et al, 2011). Stimulation of PGE$_2$ production by Polyphenolic compounds may be contributory in prevention of stomach ulcer (Sumbul et al, 2011). Protective role of Gallic acid (a polyphenolic compound) in gastropathy has been shown by many workers (Abdelwahab 2013; Pal et al, 2010).

It is supposed that the antioxidant property of polyphenols is a significant feature because reactive oxygen and /or free radicals are associated to the formation of ulcers (Shakeerabanu et al, 2011). Since the Triphala contains polyphenols, tannins and gallic acid, the ulcer healing activity observed in the this study could be accredited to these constituents (Naik et al, 2005,2006).

All biochemicals findings are confirmed by histological examination. All the effects of Triphala extract pre-treatment in Indomethacin+pyloric ligation gastric ulcer model were comparable to that of Ranitidine treatment in this model. No significant difference was found in parameters (ulcer index, mucus, TBARS, glutathione, catalase and SOD) in Triphala pre-treated group on comparison with standard drug treated group.

This shows that Triphala at the dose level of 1g/kg,oral is statistically equally effective as Ranitidine (standard drug) at the dose level of 50 mg/kg,oral in Indomethacin treated pyloric ligation stimulated preventive model of gastric ulcer.

Further studies are needed to explain the precise mechanism of action with isolated active moieties.
3.5. Conclusion

The results of the study established that Triphala pre-treatment has antisecretory action by decreasing the total acidity and volume of stomach juice. Further, the Triphala treatment presents cytoprotection by intensifying mucus wall thickness (barrier mucus) and by reducing the ulcer index.