CHAPTER 3

STUDY OF AVERRHOA BILIMBI L.FRUIT EXTRACT FOR ACETIC ACID INDUCED ULCERATIVE COLITIS IN WISTAR RATS

3.1 INTRODUCTION

Inflammatory Bowel Disease (IBD) is a chronic relapsing inflammatory condition of gastrointestinal tract with an unknown etiology. Environment, microbes, immunologic and genetic factors are routes to the pathogenesis of IBD. Crohn’s disease (CD) and Ulcerative colitis (UC) are the two forms of IBD. CD, a granulomatous inflammation leads to fistula formation, affecting large and small intestine. UC is a chronic abscess establishment restricted to colonic mucosa (Podolsky 2002) which may emanate in to colorectal cancer. The incidence of the UC and crohn’s disease increased drastically in the past 50 years in the western region of the world and it has increased from 14 to 200 in 10000 individuals. The conversion rates of the UC to colon cancer is also alarming with in different time periods (Rogler 2014). An imbalance in the levels of tumor necrosis factor-α (TNF-α), Interleukins IL-1b, IL-6, and myeloperoxidase levels and GM-CSF was found to play a key role in modulating inflammation in colon (Sakthivel and Guruvayoorappan 2014). The alterations in IL-1, IL-2 and TNF-α leads to production of IL-8 which is a potent factor for inflammation. The presently used synthetic medicines to treat UC such as Aminosalicylates, Sulfasalazine and Infliximab are having adverse side effects (Venturi et al.1999). Infliximab
treated patients had disseminated tuberculosis and pneumonia. Serious infections like aspergillosis, coccidioidomycosis, candidiasis and pneumocystis have also been observed in the Infliximab treated patients. It is clearly evident that the use of cortico steroids and immunomodulators increases the risk of infectious complications (Richard et al. 2008). The inducible nitric oxide-synthase (iNOS), cyclooxygenase-2 (COX-2) are the major elements involved in the ulcerative colitis. Under normal physiological conditions the nitric oxide is produced from L-arginine in fundamental forms of eNOS and nNOS, which plays a critical role in cytoprotection of large intestine. The COX-1 and COX-2 are responsible for the PGE2 production and proper maintainance of the cell integrity. The halting of iNOS and COX-2 enhances the cyto-protective nature of the UC. The combined blocking of both iNOS and COX2 reduces the inflammation, which controls oxidative stress and enhance the antioxidant nature against the free radicals (Sklyarov et al. 2011).

*Averrhoa bilimbi* L. fruit extract being rich in Vitamin C and other minerals (Bhaskar and Shantaram 2013) is reported to have strong antioxidant property (Sabiha et al. 2012; Hasanuzzaman et al. 2013). The UC is more associated with oxidative damage to the tissues of colon (Darren et al. 2003) leading to genetic instability, aberrant methylation and carcinogenesis in humans. The traditional uses (used against cold, cough, dermatological diseases, and fever), phytochemicals present and ethnopharmacology studies of *Averrhoa bilimbi* L. (Xavier et al. 2014) encouraged us to study its protective and therapeutic effects on UC in experimental Wistar rats. Rats treated with acetic acid were used in this study to induce UC, which resembles human UC in histopathology.
The treatment dose used in this study was selected based on previously done *in vivo* drug toxicity study report which stated that oral administration of the fruit homogenate up to a dose of 1g/kg for upto 15 days, did not show any toxic symptoms in rats (Savithri et al. 2009). *Averrhoa bilimbi* L. fruit is an edible fruit and people from Kerala region use this in pickle preparation and in fish curry. In this current study we have used minimum concentration 50mg/kg/bwt and 100 mg/kg/bwt for the anti-ulcerative colitis studies. In previous studies also the *Averrhoa bilimbi* L. fruit juice of 250 mg/kg/bwt and 500 mg/kg/bwt was used in the intoxicated wistar rats and showed increased antioxidant activity in blood and tissue. Based on the previous reports the LD50 of the *Averrhoa bilimbi* L. fruit extract must be more than 5000 mg/ kg bwt. The rats treated with *Averrhoa bilimbi* L. fruit extract up to 5000 mg/kg bwt did not show any morphological changes and toxic effects (Farah et al. 2014) and hence, the drug dose used in this study is much below the LD 50 value and is quite acceptable minimal doses for *in vivo* studies as per literature.

### 3.2 NEED FOR THE STUDY

In the previous chapter we explained the presence of phytochemicals with more phenolic content and this phenolic content is responsible for the antioxidant property such as Hexadecanoic acid, Eruic acid, and Chimarine D and can act as anti-inflammatory agent. Based on this, further we analysed the effect of *Averrhoa bilimbi* L. fruit extract on the ulcerative colitis which is an anti-inflammatory disorder in colon.
3.3 STUDY HYPOTHESIS

We propose in this study that the presence of more phenolic compounds in the fruit extract of *Averrhoa bilimbi* L. which are having antioxidants potency may have anti-inflammatory activity and it has been evaluated *in vivo* in this study.

3.4 MATERIALS AND METHODS

3.4.1 Chemicals and reagents

All the chemicals used for this experiment were of analytical grade. Interleukins IL-1b and IL-6 were purchased from KOMBA Biotech (Seoul, Korea), COX-2 kit was purchased from the Blue Gene Biotech (Shanghai, China). Tumor necrosis factor (TNF-α) and iNOS were obtained from the USCN Life science (Wuhan, Hubei, PRC). Sulfasalazine standard drug was procured from Wallace Pharmaceuticals, Goa, India.

3.4.2. Study sample

The ripe fruits of *Averrhoa bilimbi* L. were cleaned, shade dried, ground into coarse powder and 25 gms of it was extracted with methanol (95%v/v) using the soxhlet apparatus and rotary evaporator. The yield of the *Averrhoa bilimbi* L. fruit extract was 12% as it is a juice edible fruit. The thick semi solid extract that was obtained was stored at 2–4 °C for study.
3.4.3. Animals

Male Wistar rats of 7–8 weeks of age with 150–180gms, procured from the Kerala Veterinary and Animal Sciences University Thrissur, Kerala, India were maintained in a controlled sterile environment such as, at 12 h’ light/dark cycle, constant temperature (25°C) and 50% relative humidity. Experimental rats had ad libitum access to standard diet pellets (Sai Durga Feeds, Bangalore, India) and filtered water. All experiments were conducted by following the rules and regulations of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India, after getting the approval from Institutional Animal Ethics Committee (No: IAEC/KU/BT/15/08), Karunya Institute of Technology and Sciences.

3.4.4. Experimental design

Twenty rats were divided into five experimental groups of four rats each as follows: Group 1: Normal Rats; Group 2: Ulcerative Colitis control; Group 3: Ulcerative Colitis + *Averrhoa bilimbi* L. (50 mg/kg/bwt); Group 4: Ulcerative Colitis + *Averrhoa bilimbi* L. (100 mg/kg/bwt); Group 5: Ulcerative Colitis + Standard drug Sulfasalazine (100 mg/kg/bwt). In the normal group (Group 1), rats were treated with normal saline for 6 consecutive days. After one day fasting, except group 1 rats all other experimental group rats were induced for UC with single dose of 2 mL of 3% acetic acid which introduced by using polyethylene tube of 2 mm in diameter through the rectum into the colon region up to 8 cm. The rats were kept in supine Trendelenburg position for 30s to hinder the intracolonic instillate leakage (Millar et al. 1996). Experimental rats group 3 (50 mg/kg/bwt) and 4 (100 mg/kg/bwt) were treated with *Averrhoa bilimbi* L. fruit extract. Group 5 was treated with standard drug Sulfasalazine (100 mg/kg/bwt) (Sakthivel and Guruvayoorappan 2016). All these
drugs were given intraperitonially for 6 consecutive days. The rats were sacrificed after 24 h of induction and the spleen and colon tissues were dissected out for analysis. The weight of the spleen and colon tissues were evaluated to assess the gain in weight due to inflammation. The dissected colonic specimens were washed with ice cold PBS (pH 7.2) and stored in 10% formalin for the Immunohistochemistry, Histopathology and for morphologic studies. A portion of colon tissue from each rat was homogenized for the assay of inflammatory markers such as TNF-α, IL-1b, IL-6, iNOS and COX-2 using ELISA method.

3.4.5 Evaluation of ulcerative colitis

3.4.5.1 Determination of the Colon and Spleen weights

After sacrifice, the spleen was collected from every group rats and their weights were measured as it is vital organ involved in inflammatory process. A 5 cm segment of distal colon, 3 cm proximal to anus was resected to determine the weight of the colon. Weight of the colon and spleen were measured and expressed in milligrams per centimetre (mg/cm) and the mean ± SD for every group was calculated.

3.4.5.2 Analysis of the Colon tissue morphology by visual scoring

The intensity of ulcerative colitis was assessed visually and imaged by an independent observer who was ignorant to the treatment. About 10 cm distal portion of each rat colon was removed after sacrifice. The colon was dissected longitudinally and washed with normal saline to remove fecal materials. The visual assessment on the inflammation status was done based on the features of colon using a scoring pattern (Sakthivel et al. 2012): 0 is no visible change (no damage), 1 is with Hyperemia where water oozes out, 2 is
lesions in diameter of 1 cm without hyperemia, 3 is Ulceration with inflammation at two sites, 4 is major sites of inflammation > 1 cm along the organ with redness, 5 is major sites of inflammation > 2 cm along the organ with redness, 6 is major sites of inflammation > 3 cm along the organ with redness, 7 is major sites of inflammation > 4 cm along the organ with redness, 8 is major sites of inflammation > 5 cm along the organ with redness, 9 is major sites of inflammation > 6 cm along the organ with redness, swelling and bleeding.

3.4.5.3 Histopathological evaluation of UC

The histopathological analysis was done to observe the inflammatory changes in the colon specimen fixed in 10% formalin in PBS and embedded in paraffin. About 4µm thick sections of colon were placed on glass slide, deparaffinised and hydrated with decreasing percent ethanol. Then the sections were stained with Eosin and Hematoxylin and this, Hematoxylin stains acidic molecules as blue and eosin stains basic materials as red to pink. Then 70% to 100% ethanol was used to dehydrate the slides and for the cleaning xylene was used. Then the tissue sections were mounted with DPX (mount media) and were observed under light microscope. All sections were analysed and interpreted by a certified histopathologist.

3.4.5.4 Immunohistochemical study for the expression of COX-2 and iNOS

The paraffin embedded colon tissue was used for this study. Briefly, 4µm thick colon sections were fixed on slides, dewaxed and hydrated. These sections were treated with 3% bovine serum albumin (buffered blocking solution) for 15mins. Then the sections were incubated with primary antibody for COX-2 and iNOS at a dilution of 1:400 in PBS for 24 h at room
temperature, followed by washing with PBS. Again the sections were incubated with PBS along with the secondary biotinylated antibody for 30 minutes and treated with substrate for peroxidase. Then, the sections were incubated with 3, 3-diaminobenzidine solution for 10 mins at room temperature in darkness followed by Tris-HCl wash. The sections were then counter stained with Hematoxylin and the section was dehydrated. The sections were coverslipped with mounting medium for the final observation under light microscope. The expression of COX-2 and iNOS was identified by the brown colour in tissue sections as positive cells. The expression levels of COX-2 and iNOS were interpreted by a certified histopathologist.

### 3.4.5.5 Determination of the effect of *Averrhoa bilimbi* L. on inflammation mediatory cytokines

The colon tissue of 3 to 5g was cut, cleaned and homogenized in a mortar with buffer and then centrifuged at 5000 rpm. The obtained supernatant of the colon tissue homogenate was then used for measuring the levels of TNF-α, IL-1b, IL-6, iNOS and COX-2 using specific antibody for cytokines by the standard sandwich Enzyme-Linked Immunosorbent Assay (ELISA) with the ELISA kit according to the manufacture’s instruction. The colour development was measured at 450 nm. From the OD values the level of inflammatory markers was calculated using standard curves.

### 3.5 STATISTICAL ANALYSIS

Mean ± SD was used for all data and value expression. A one-way analysis of variance (ANOVA) used for statistical analysis followed by


Dunnett’s test by the Graphpad InStat version 3.0 (Graphpad Software, San Diego, CA).

3.6 RESULTS

3.6.1 Weight of Colon and Spleen

A significant increase in colon weight (P≤0.001) was observed in UC control group when compared with normal colon as a result of acetic acid induced inflammation. Similarly, spleen weight also increased in UC control as a consequence of increased inflammation reactions. In groups 3 and 4 due to the treatment of *Averrhoa bilimbi* L. fruit extract (50 and 100 mg/kg bwt) the colon and spleen weight were significantly lowered (** p ≤ 0.01; ***p ≤ 0.001) respectively as observed in standard drug treatment in group 5 (Figure 3.1).

Table 3.1 Effect of *Averrhoa bilimbi* L. (50 mg/kg.b.wt and 100 mg/kg.b.wt) on colon wet weight and spleen weight

<table>
<thead>
<tr>
<th>S.No</th>
<th>Group</th>
<th>Colon weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Group I</td>
<td>54.5 ± 3.41</td>
</tr>
<tr>
<td>2</td>
<td>Group II</td>
<td>169 ± 3.74***</td>
</tr>
<tr>
<td>3</td>
<td>Group III</td>
<td>127 ± 3.55***</td>
</tr>
<tr>
<td>4</td>
<td>Group IV</td>
<td>106.25 ± 3.96***</td>
</tr>
<tr>
<td>5</td>
<td>Group V</td>
<td>119.5 ± 4.2***</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>S.No</th>
<th>Group</th>
<th>Spleen weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Group I</td>
<td>0.955 ± 0.13</td>
</tr>
<tr>
<td>2</td>
<td>Group II</td>
<td>1.655 ± 0.26***</td>
</tr>
<tr>
<td>3</td>
<td>Group III</td>
<td>1.3535 ± 0.16b***</td>
</tr>
<tr>
<td>4</td>
<td>Group IV</td>
<td>1.13 ± 0.29c***</td>
</tr>
<tr>
<td>5</td>
<td>Group V</td>
<td>1.35 ± 0.31d***</td>
</tr>
</tbody>
</table>
Figure 3.1 Effect of *Averrhoa bilimbi* L. (50 mg/kg. b.wt and 100 mg/kg.b.wt) on colon wet weight and spleen weight

Values are expressed in mean ± SD. a Control vs normal group (**p ≤ 0.001), b control vs Group 3 (**p ≤ 0.01), c control vs Group 4 (**p ≤ 0.001), d control vs Group 5 (***p ≤ 0.001).

### 3.6.2 Morphologic observation of colon tissue

Colonic tissue damage was observed (Figure .33.A) in group 2 due to the induction of UC with 3% acetic acid and it was decreased in group 3 and 4 due to the treatment with *Averrhoa bilimbi* L. fruit extract (50 and 100 mg/kg b wt), similar to group 5 treated with standard drug. The scoring for morphologic change was given (Sakthivel et al. 2014) in Table 3.2 and Figure. 3.3B. UC control group 2 colon appeared as intense edematous inflammation with redness after 24 h accounting to a high score of 9 when compared with normal. The *Averrhoa bilimbi* L. fruit extract treatment in group 3 and 4 meliorated the effect of acetic acid as indicated by the reduced score of 7 ± 0.72 and 5 ± 0.95 respectively (**p ≤ 0.01), corresponding to the dose of treatments. The same results were obtained in group 5 rats treated with standard
drug (100 mg/kg bwt) also with a score of 7. Surprisingly the group 4 treated rat showed better curative effects (score 5) than that of group 5 rats (score 7).

Table 3.2 Effect of *Averrhoa bilimbi* L. on Ulcerative macroscopic scoring

<table>
<thead>
<tr>
<th>S.no</th>
<th>Group</th>
<th>Macro scoring</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Group I</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Group II</td>
<td>10 ± 0.81</td>
</tr>
<tr>
<td>3</td>
<td>Group III</td>
<td>7 ± 0.72</td>
</tr>
<tr>
<td>4</td>
<td>Group IV</td>
<td>5 ± 0.95</td>
</tr>
<tr>
<td>5</td>
<td>Group V</td>
<td>7 ± 0.85</td>
</tr>
</tbody>
</table>

Figure 3.2 Appearance of colon tissue in acetic acid induced and treated rats

1. Colon damage was not observed in normal group.
2. UC control tissue with intense reddish and edemators
3. and 4. Treated colon showing reduction in inflammatory changes
4. Standard drug treated colon in showing a good prevention of inflammatory edema and redness.
Values are expressed in mean SD. a Control vs normal group (**p ≤ 0.001), b control vs Group 3 (**p ≤ 0.001), c control vs Group 4 (**p ≤ 0.001), d control vs Group 5 (**p ≤ 0.001).

3.6.3 Effect of *Averrhoa bilimbi* L. fruit extract on the histopathology of UC

A high level of transmural necrosis with infiltration of inflammatory cells in the layers of bowel wall, edema and loss of epithelial cells were observed in the UC control group (Arrow in figure 3.5;2). The *Averrhoa bilimbi* L. fruit and standard drug sulfasalazine treated groups have shown a significant reduction in the cell damage, mucosal and edema injury (figure 3.5;3; 3.5;4 & 5). Infiltration of Inflammatory cells also was inhibited by the *Averrhoa bilimbi* L. fruit and sulfasalazine when compared with the control group as shown in Figure 3.5.
Figure 3.4 Histopathology of the colonic mucosal section

Group 1 is with normal mucosa and epithelial cells. Group 2 with Ulcerative colitis control group which shows mucosal ulceration and necrosis with infiltration of cells as a mark of inflammation. High level of transmural necrosis in the layers of bowel wall and loss of epithelial cells was observed in the control group. The Groups 3, 4 and 5 shows reduced ulceration and inflammation showing decreased cell damage and mucosal injury. Infiltration of inflammatory cells also was inhibited by the *Averrhoa bilimbi* L. fruit extract and sulfasalazine when compared with the control group.

3.6.4 The expression levels of iNOS and COX-2 in colon tissue by Immunohistochemistry

Normally the expression of iNOS (inflammatory mediator) is seen in neutrophils and smooth muscle cells with a sparse distribution in epithelial cells of normal animals. The COX-2 expression is found in the epithelial surface and mononuclear cells of lamina and propria of mucosa of normal animals. The colon immunohistochemical examination revealed that the iNOS
and COX-2 expressions were raised in acetic acid induced UC control group when compared with the normal group. i.e., *Averrhoa bilimbi* L. and sulfasalazine treatment have reduced the expression levels of iNOS and COX-2 in the acetic acid induced Ulcerative colitis compared to the UC control group as shown in Figure 3.6 and 3.7.

(Magnification 40x)

**Figure 3.5 Immunohistochemical expression of iNOS**

1. Normal group with out treatment.
2. The colon immunohistochemical examination revealing an increased iNOS level in acetic acid induced UC control group.
3-4 The colon of groups showing a reduced level of iNOS expression when compared with control group.
5. The standard drug is also showing reduced levels of iNOS and COX-2 expression.
2. COX-2 level is increased in UC control group.
3 – 4 Treatment with *Averrhoa bilimbi* L. (50 mg/kg.b. wt and 100 mg/kg.b.wt) respectively showing decreased COX-2 levels.
5. Sulfasalazine (100 mg/kg.b.wt) treatment showing decreased expression level of COX-2 compared with control group 2.

### 3.6.5 Effect of *Averrhoa bilimbi* L. on the inflammatory mediators

The quantitative evaluation of the levels of inflammatory mediators such as TNF-α, IL-1β, IL-6, iNOS and COX-2 by ELISA technique revealed the effect of *Averrhoa bilimbi* L. fruit extract. We have observed an increased cytokines level in the colon after acetic acid induction in the control group. The levels of inflammatory mediators observed in UC control group 2 were TNF-α (107.75 ± 2.9 pg/mg), IL-1b (102.75 ± 3.5 pg/mg), IL-6 (124 ± 3.6 pg/mg) iNOS (83.25 ± 4.3 ng/mg) and COX-2 (100.5 ± 2.6 ng/mg). These levels were
found to be decreased significantly (** p ≤ 0.01) in the treated groups i.e in group 3 and 4 the levels of TNF-α, IL-1β, IL-6 iNOS and COX-2 were 45.5 ± 2.6 pg/mg, 62 ± 4.2 pg/mg, 54.25 ± 3.7 pg/mg, 31.5 ± 2.08 ng/mg and 44.75 ± 2.87 ng/mg respectively. Group 5 rats treated with the standard drug also has shown decreased levels of the TNF-α, IL-1β, IL-6, iNOS and COX-2 as shown in Figure 3.8

Figure 3.7 Effect of *Averrhoa bilimbi* L. fruit extract (50mg/kg.b.wt and 100mg/kg.b.wt) on the levels of cytokines IL-1β, IL-6, TNF-alpha and inflammatory mediators iNOS, COX-2

Values are expressed in mean±SD. a control vs normal Group (** p ≤ 0.01), b control vs Group 3 (** p≤ 0.01), c control vs Group 4 (** p≤ 0.01), d control vs Group 5 (** p≤ 0.01).
3.7 DISCUSSION

UC is a chronic relapsing disorder that causes damage to the colonic mucosa and the pathology of such Inflammatory Bowel Diseases (IBD) is unknown. Oxidative stress plays a major role in UC patients. The colon tissue in UC undergoes tissue damage such as granular, hyperaemic mucosa and the colon region becomes congested with the bowel leads to the perforation (Rhodes 2000) with a prominent inflammation. Ulcers are the sores formed on the protective mucus layer, where the mucus layer become ineffective. The intense ulcerative colitis can damage up to the extended part of the lamina propria (Shergill Amandeep et al. 2015). This involves the inflammation which produces the noxious compounds in surrounding regions and leads to DNA damage, transformation of cells or proceeds further towards transformation of cells to cell proliferation, which increase the chances of causing colon cancer. The inflammatory changes lead to the production of free radicals which have the ability to cause p53 mutation leading to cancer. The iNOS and COX2 expressions are high in the inflammatory site, which play a crucial role in the induction of the UC (Wong and Harrison 2001). The UC is a type of chronic inflammation in which cellular homeostasis is deregulated and can cause colon cancer. The free radicals produced during the chronic inflammation can cause post translational changes and gene mutations, which are the major cause in the initiation of cancer (Hussain et al. 2003). In this study, we have used 3% of 2mL of acetic acid to induce UC as per the available literature (Gulgun tahan 2011).

Plants have the potential to promote repair mechanism in a natural way with fewer side effects (Xavier et al. 2012). In this current research, we have evaluated the medicinal effect of *Averrhoa bilimbi* L. on the induced UC in rats. *Averrhoa bilimbi* L. fruit is rich with phytochemicals which are having
potent antioxidant activity either by scavenging the reactive molecules or by enhancing antioxidant molecules and enzymes. The *Averrhoa bilimbi* L. fruit is rich source of Vitamin C which is a potent antioxidant. Biochemical analysis report of *Averrhoa bilimbi* L. shows the presence of several minerals including nitrogen, nitrate, boron, potassium, calcium, phosphorus, sulphur, copper, manganese, molybdenum, magnesium and zinc (Dangat et al. 2014). Boron can be used effectively for the wound healing process (Nielsen 2002). The nitrate helps in controlling the iNOS in the body against scavenging free radicals (Bauerova and Bezek 2000). Copper, Zinc and Manganese can be used in the modulation of the wound healing keratinocytes (Tenaud et al. 1999).

*Averrhoa bilimbi* L. fruit extract exhibited a significantly lowered ulcer scoring, the wet weight of colon tissue and the spleen weight in our study which is indicating its anti-ulcer property. The visual scoring of ulcerative colitis, changes in the weight of colon, spleen and the histological changes in colon tissue have shown this potent medicinal effect of *Averrhoa bilimbi* L. fruit extract over UC. The treatment with *Averrhoa bilimbi* L. fruit extract in our study has also optimally maintained the levels of inflammatory mediators, TNF-α, IL6, IL 1b, iNOS and COX-2 in acetic acid induced UC (figure 3.8). In UC, the cytokines TNF- α, IL-1b, IL-6, are usually enhanced due to inflammation in the colon. In IBD Pathogenesis TNF- α act as a major component and a study revealed that the restriction of TNF- α suppresses the Colitis and Colorectal Cancer (Popivanova et al. 2008). It was also reported that in IBD patients, the TNF-α was expressed abundantly in the gut (Nakamura et al. 2006) and is involved in anti-apoptosis process (Tabassum and Saima 2010). TNF-α and IL1b activates mesenchymal cells and leads to the inflammatory response and fibrosis (Stallmach et al. 1992). IL1b was found to initiate anion secretion and augments bradykinin, H₂O₂, histamine-induced epithelial chloride section and played vital role in intestinal inflammation (Eigler et al. 1997). The
patients diagnosed with IBD showed a high production of IL-6 in serum and intestinal lamina propria (Rahbar et al. 2003). All these cytokines have elevated level in the colon of UC control group rats in our study (Figure 3.8) which attracts the inflow of the immune cells such as neutrophils and mono nuclear cells. The Colon in control group showed the infiltration in our study and it has been reduced by the treatment of *Averrhoa bilimbi* L. fruit extract. *Averrhoa bilimbi* L. fruit extract was also found to exhibit an anti-inflammatory activity by down regulating the COX-2 and i-NOS levels in the colon as revealed by the ELISA quantification as well as immunohistochemistry findings in our study (Figure 3.6 and 3.7). COX-2 and i-NOS enzymes are the major enzymes expressed at the site of injury (Kankuri Esko et al. 1999). The studies related to COX1 and COX2 alteration in mice indicated the importance of these inflammatory mediators in carcinogenesis, in which COX2 has the major role in facilitating carcinogenesis (Howard et al. 2002). In the inflammed tissues the high levels of prostaglandins were found due to the stimulation by the nitric oxide. The increase in the COX-2 and iNOS inducible enzymatic pathway leads to the production of prostaglandins and nitric oxide, which are known to cause inflammation and major tissue damage. The eNOS/NO of NO-synthase provide cytoprotective mechanism and the disturbance in the iNOS/NO causes the inflammation and ulcer condition (Needleman and Manning 1999).

Inflammatory mediators normally exhibits defence mechanism against injury, infection and inflammation in pathological conditions. The Inflammatory mediators (iNOS and COX-2) over expresses or up regulated during inflammation (Weon-Jong et al. 2009). and the over expressed iNOS and COX-2 were found to be controlled by the NF-kB which is a pro-inflammatory transcription factor (Simmons et al. 2004). The phytochemicals from the plant source have the ability to control the inflammation and maintain the level of the iNOS and COX-2 levels (Mattace et al. 2001). The ulcerative
colitis induced tissue injury in our study also has shown an elevated expression of iNOS and COX2 in colon tissue of UC control rats which were reduced in treatment with *Averrhoa bilimbi* L. fruit extract.

Our study results, thus implies that the *Averrhoa bilimbi* L. fruit extract has a protective and curative effects on Ulcerative Colitis. The triterpenoids (phytochemicals) are able to suppress inducible iNOS and COX-2, the inflammatory mediators. By this evidence plant products with rich phytochemicals can maintain the optimum levels of iNOS and COX-2 (Najoo et al. 1998). Our plant under study *Averrhoa bilimbi* L. fruit also has shown the presence of triterpinoids and other phytochemicals such as phenolic compounds (2-Hexadecanol, 1-Heneicosanol, n-Tetracosanol,1-Heptacosanol, 2-[(N,N-Dimethylamino) methyl]-4- fluorophenol, Cyclohexanone, 2,3,4-trihydroxy-6-methyl, Guanosine and D-glycero-D-manno-Heptitol), flavonoids (4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl, 2-Methylacetophenone-dioxolane and Desulphosinigrin), Alkaloids (2,2-Diethyl-N-ethylpyrrolidine, Hygroline, 2-Cyclohexylpiperidine and 2-Pentenal), saponins (2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one) and Terpenoids (Squalene) which may be the reason for its anti-inflammatory action in UC as evidenced in induced rats.