Chapter 3. Evaluation of the protective effect of Bauhinia tomentosa on experimental ulcerative colitis.

3.1 Introduction

Inflammatory bowel disease (IBD) is a chronic inflammatory disorder involving primarily the gastrointestinal tract and also includes Crohn’s Disease and Ulcerative Colitis. The pathology of IBD is characterized by polymorphonuclear leukocyte (PMNL) infiltration, oedema, erythema and ulceration (Frettland et al., 1990). Oxidative stress has been implicated in the pathogenesis of Ulcerative Colitis (Keshavarzian et al., 1990; Kitahora et al., 1988). Excess production of reactive oxygen metabolites such as superoxide, hydroxyl radical, hydrogen peroxide, hypochlorous acid and oxidant derivatives N-chloramines, are detected in the inflamed mucosa and are reported to be pathogenic in inflammatory bowel disease (Keshavarzian et al., 1992). Sustained production of reactive oxygen metabolites during colonic inflammation may overwhelm the endogenous antioxidant defense system that regulates their production leading to oxidative injury (Blau et al., 1999).

Although ulcerative colitis etiology is largely unknown but the current literature suggest that multiple immune, genetic, and environmental factors influence both the initiation and progression of colitis (Fiocchi et al., 1998). There is evidence for an intense local immune response associated with recruitment of lymphocytes and macrophages followed by release of soluble cytokines and other inflammatory mediators. Subsequent activation of these cells causes a self-augmenting cycle of cytokine production, cell recruitment and inflammation (Sartor, 1997; Shanahan, 2001). This uncontrolled immune system activation results in a sustained massive production of cytokines such as tumor necrosis factor (TNF)-α and interleukins (IL-1β and IL-8) (Ogataa and Hibi, 2003). In addition to cytokines, leukotrienes, thromboxane, platelet-activating factor, nitric oxide and reactive oxygen species are also released from activated mucosal cells (Podolsky, 1991; Woywodt et al., 1999).
Most of the current therapies for inflammatory bowel diseases involve treatment with glucocorticosteroids and 5-aminosalicylic acid (Podolsky, 1991; Strober et al., 1998). Immunosuppressive drugs have also been used to control severe illness, regardless of the more serious complications and toxic side effects associated with them (Shanahan, 2001). Although many types of treatments have been proposed and clinically proven, additional therapeutic approaches are needed because many patients do not respond to the currently available options which demonstrate significant side effects.

Plants and plant based drugs have been used to treat various diseases from time immemorial which is proved to be less toxic and free from side effects. The genus Bauhinia belongs to Fabaceae family, subfamily Caesalpiniaeae which comprises about 300 species distributed in tropical and subtropical regions. Several therapeutic properties are attributed to Bauhinia species, which includes anti-ameboic, anti-diabetic, analgesic, anti-rheumatic and hypocholesteromic activities (Viana et al., 1999). Bauhinia tomentosa is a shrub prevalent among south India and is widely used in ayurvedic preparations. This plant has been reported to possess antioxidant (Khalil et al., 2008), gastroprotective (Gonzalez-Mujica et al., 2003), hepatoprotective (Bodakhe and Ram, 2007) and antimicrobial effect (Gopalakrishnan and Vadivel, 2011). Aderogha et al., (2008) reported that the ethanolic extract of B.tomentosa leafs contain kaempferol-7-O-rhamnoside, kaempferol-3-O-glucoside, quercetin-3-O-glucoside and quercetin-3-O-rutinoside. In the present study we had evaluated the protective effect of B.tomentosa during experimental ulcerative colitis and the possible mechanism of action.
3.2 Materials and Methods

3.2.1 Preparation and administration of plant extract

*Bauhinia tomentosa* leaves were collected from Coimbatore. The plant specimen was authenticated from Botanical survey of India, TNAU campus, Coimbatore (BSI/SRC/5/23/2011-12/Tech-756). *B. tomentosa* leaves were shade-dried, powdered and extracted (10g) with 100ml of 70% methanol by stirring overnight. The supernatant was collected after centrifugation at 5,000 rpm for 10 min. The solvent was removed by evaporation and yield of the extract was found to be 8%. For *in vivo* studies, the extract was re-suspended in 1% gum acacia and administered at a concentration of 10mg/Kg Body weight (i.p.) respectively.

3.2.2 Animals

Adult male Wistar rats (200-250g) (n=24) were purchased from Kerala Veterinary and Animal Sciences University, Mannuthy, Thrissur. The animals were kept under controlled conditions and fed with normal mouse chow (Sai Durga Feeds, Bangalore, India) and water *ad libitum*. All animal experiments were performed after getting prior approval from Institutional Animal Ethics Committee (Approval No. IAEC/KU/BT/12/017), Karunya University, Coimbatore.

3.2.3 Chemicals

Sulfasalazine was procured from Wallace pharmaceuticals, Goa, India. Lactate dehydrogenase (LDH) assay kit was procured from Bio vision, California, USA. Myeloperoxide (MPO), Tumor Necrosis Factor (TNF-α) and inducible Nitric Oxide Synthase (iNOS) kits were purchased from USCN Life science Inc, Houston, USA. All other chemicals used were analytical reagent grade.
3.2.4 Experimental design

Rats were divided into 4 groups (n=6 per group). Group I were kept as normal and received no treatment. Group II, III and IV were subjected to the induction of ulcerative colitis by intra-colonic injection of 2ml acetic acid (3% v/v). Group II served as ulcerative colitis control group. Group III was treated with standard drug sulfasalazine (100 mg/kg, B.wt) intra-peritoneally and Group IV were treated with B.tomentosa extract (10 mg/kg, B.wt (i.p.) for 5 consecutive days before induction of ulcerative colitis.

3.2.5 Induction of colonic inflammation in rats

All the animals (except group I) were kept fasting overnight, with access to water ad libitum and anesthetized by ether inhalation before induction of colitis. 2 ml of acetic acid (3%, v/v) were infused for 30s using a polyethylene tube (2mm in diameter), inserted through rectum into the colon up to a distance of 8 cm. 24h later, blood and colon were collected after sacrificing the animals. For separation of serum, blood samples were centrifuged at 3500rpm (10min) and the serum collected were stored at -20°C. Portions of colon specimens were dissected out, washed with ice cold PBS (pH 7.2) and kept in 10% formalin for microscopic and histopathological studies. The remaining portion of colon specimens were used for biochemical studies.

3.2.6 Assessments of colitis

3.2.6.1 Macroscopic scoring

For each animal, the distal 10 cm portions of the colon were removed and cut longitudinally, cleaned with physiological saline to remove fecal residues. Macroscopic inflammation scores are assigned based on the clinical
features of the colon using an arbitrary scale ranging from 0-4 as follows: 0 (no macroscopic changes), 1 (mucosal erythema only), 2 (mild mucosal oedema, slight bleeding or small erosions), 3 (moderate oedema, slight bleeding ulcers or erosions) and 4 (severe ulceration, edema and tissue necrosis) (Miller et al., 1996).

3.2.7 Biochemical assays

The colon tissue were homogenized in 10mmol Tris-HCl buffer (pH 7.4) and the homogenate were used for the measurement of Nitric oxide (NO), Myeloperoxidase (MPO), Lipid peroxidation (LPO), reduced glutathione (GSH) and TNF-α.

3.2.7.1 Determination of the effect of B.tomentosa on serum Nitric oxide (NO) level

Serum NO level were estimated by using Griess reaction according to the standard method described by Green et al., 1982.

3.2.7.2 Determination of the effect of B.tomentosa on colon lipid peroxidation

Lipid peroxidation, an indicator of mucosal injury induced by reactive oxygen species were measured as described by Ohkawa et al., 1979. Briefly, 0.5ml of colon tissue homogenate is mixed with 2ml of TBA reagent containing 0.375% TBA (Thiobarbituric acid), 15% trichloroacetic acid and 0.25 N HCl. The mixture was then boiled for 15min, cooled and centrifuged (2000 rpm; 15 min). Absorbance of the supernatant was measured at 532nm. TBARS concentration was calculated using 1,3,3,3 tatra-ethoxypropane as a standard. The results were expressed as µmol/g wet tissue weight.
3.2.7.3 Determination of the effect of *B. tomentosa* on colonic Glutathione (GSH) level

The Colon GSH level was determined using the standard method explained by Moron et al, 1979. GSH in colonic tissue homogenate were allowed to react with Ellman’s reagent (5, 5-Dithio-2-nitrobenzoic acid) in phosphate buffer saline (PBS, pH 8.0) and the absorbance was measured at 412nm. GSH concentration were calculated using the standard GSH. The results are expressed as nmol/g wet tissue weight.

3.2.7.4 Determination of the effect of *B. tomentosa* on Superoxide dismutase (SOD) activity

The determination of SOD were measured by using standard method of Marklund and Marklund (1979). Enzyme activity was calculated from the inhibition of the reduction of NBT (Nitroblue Tetrazolium). The tissue homogenate were incubated with 0.2ml EDTA/NaCN, 0.1ml NBT, 0.05ml riboflavin and the absorbance was measured at 560nm before and after illumination. The values are expressed as nmol per milligram protein.

3.2.7.5 Determination of the effect of *B. tomentosa* on Glutathione Peroxidase activity

The GPx activity was measured using the standard method of Paglia and Valentine (1967). The mixture of 0.2 ml Tris-HCl buffer (pH 7), 0.2 ml EDTA, and 0.1 ml sodium azide and tissue homogenate were prepared. GSH (0.2 ml) was added to this followed by the addition of H₂O₂ (0.1ml). The contents were mixed well and incubated at 37°C for 10 min. The reaction was arrested by the addition of 0.5ml of 10% TCA. The tubes were centrifuged and the absorbance of the supernatant was measured at 340nm. The result was expressed as µmoles of GSH utilized/min/mg protein.
3.2.7.6 Determination of the effect of *B. tomentosa* on the tissue TNF-α, iNOS and MPO level

The colon tissue homogenate were used for the measurement of TNF-α, iNOS, and MPO levels according to manufacturers’ instructions (USCN Life science Inc, Houston, USA). The values were expressed as pg/mg tissue (for TNF-α) and ng/g tissue (for iNOS and MPO respectively).

3.2.8 Histopathology analysis

The Colon specimens (2 cm) collected from the animals were fixed in 10 % formalin, embedded in paraffin and cut into 4µm sections. The paraffin sections were deparafinized with xylene, hydrated and stained with hematoxylin and eosin for studying mucosal damage assessment.

3.2.9 Immunohistochemistry analysis

The colonic tissues were fixed in 4% neutral formalin, dehydrated with increasing concentrations of ethanol, embedded in paraffin, and sectioned. Sections (5µm thick) were mounted on slides, cleaned, and hydrated. The sections were treated with a buffered blocking solution (3% bovine serum albumin in phosphate-buffered saline (PBS) for 15 min. Then, the sections were co-incubated with primary antibody for COX-2 or iNOS at a dilution of 1:400 in PBS (v/v), at room temperature for 1 and 24 h respectively, followed by washing with PBS and co-incubated with secondary antibody at a dilution of 1:500 in PBS (v/v), at room temperature for 1 h. Thereafter, sections were washed as before and with Tris-Hcl 0.05 M, pH 7.66, and then co-incubated with a 3, 3’-diaminobenzidine solution in darkness, at room temperature for 10 min. The sections were washed with Tris-Hcl, stained with haematoxylin according to standard protocols, mounted with glycerin and observed in an EVOS-xl CORE light microscope (AMG, Bothell, WA).
3.2.10 Statistical analysis

The results are expressed as mean ± S.D. Statistical analysis was carried out using one-way analysis of variance followed by Dunnet’s test using GraphPad Instat (version 3.0 for Windows 95; Graph Pad Software, San Diego, CA). p-values <0.05 were considered as statistically significant. All the experiments were performed at least three times.

3.3 Results

3.3.1 Effect of *B.tomentosa* on the colon morphology, macroscopic scoring and wet weight during ulcerative colitis.

The effect of *B.tomentosa* on colon morphology is presented in Figure 3.1. Acetic acid caused severe colon mucosal injury and macroscopic edematous colon inflammation. The wet colon weight from the animals treated with *B.tomentosa* (10mg/kg B.wt.) were found to be significantly (p<0.01) decreased (125mg/cm) compared with the colitis control group (172 mg/cm) where as the standard drug Sulfasalazine (100 mg/kg B.wt.) treatment showed a wet colon weight of 145mg/cm. The wet colon weight of the normal animals were found to be 70.5 mg/cm. Similarly the macroscopic inflammation score also showed that treatment with *B.tomentosa* (10mg/kg B.wt.) could inhibit ulcerative colitis (Table 3.1).
Fig 3.1: Effect of *B.tomentosa* on colon morphology during ulcerative colitis

A) Normal  B) Ulcerative colitis control  C) UC + sulfasalazine (100mg/kg.B.wt.)  D) UC + *B.tomentosa* (10 mg/kg.B.wt.).
Table 3.1: Effect of *B.tomentosa* on the macroscopic scoring and wet weight during ulcerative colitis.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Colon wet weight/length (mg/cm)</th>
<th>Macroscopic score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>70.5 ± 4.1</td>
<td>-</td>
</tr>
<tr>
<td>Colitis control</td>
<td>172 ± 11.4</td>
<td>3.90 ± 0.10</td>
</tr>
<tr>
<td>Colitis + Sulfasalazine</td>
<td>145 ± 9.5</td>
<td>2.25 ± 0.25</td>
</tr>
<tr>
<td>Colitis + <em>B.tomentosa</em></td>
<td>125 ± 8.5</td>
<td>1.95 ± 0.30**</td>
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</tbody>
</table>

Colitis was induced using 3% of acetic acid and treated with *B.tomentosa* (10 mg/Kg. B.wt.) or standard drug sulfasalazine (100mg/kg. b.wt.). After the experimental regimen the colon was removed and the wet colon weight and the macroscopic score were noted. Values are expressed as mean ± S.D. **p< 0.01 compared with colitis alone group.
3.3.2 Effect of *B. tomentosa* on colon lipid peroxidation level during ulcerative colitis

The effect of *B. tomentosa* on the colon lipid peroxidation level is shown in Figure 3.2. Treatment with *B. tomentosa* (10 mg/kg B.wt.) significantly decreased the colon lipid peroxidation (3.39 nmol/mg protein) compared to colitis control group (6.43 nmol/mg protein). The lipid peroxidation in normal animals was found to be 2.36 nmol/mg protein.

3.3.3 Effect of *B. tomentosa* on colon GSH, SOD and GPx level during ulcerative colitis

Effect of *B. tomentosa* on Colon GSH, SOD and GPx level is shown in (Fig 3.3 to 3.5). The decreased colonic GSH in the colitis control group (33.91 nmol/mg protein) was found to be significantly (p<0.05) increased (42.37 nmol/mg protein) after *B. tomentosa* treatment (10mg/kg B.wt.). Similarly the decreased SOD (943.01 nmol/mg protein) and GPx (27.08 µg of GSH utilized/min/mg protein) level in the colitis control group was found to be increased to 1023.48 nmol/mg protein and 42.34 µg of GSH utilized/min/mg protein respectively. The normal level of colon GSH, SOD and GPx was found to be 60.53 nmol/mg protein, 1239.20 nmol/mg protein and 59.24 µg of GSH utilized/min/mg protein respectively.

3.3.4 Effect of *B. tomentosa* on the NO level and iNOS expression during ulcerative colitis

Nitric oxide (NO) production and inducible nitric oxide synthase (iNOS) expression in colonic tissue appears during active ulcerative colitis, and these are present in excess to play the proinflammatory role (Guslandi 1998). Consequently, we estimated the effect of *B. tomentosa* on NO production and iNOS expression in the colon during ulcerative colitis.
Effect of *B.tomentosa* (10mg/kg,bwt.) and sulfasalazine (100mg/kgb.wt) on colonic tissue TNF-α level in rats with acetic acid-induced ulcerative colitis (mean of 6 animals±SD). As compared with acetic acid alone treated control group (one-way ANOVA followed by Dunnett’s t test), **p < 0.01. 

Fig 3.2: Effect of *B.tomentosa* on colon lipid peroxidation (LPO) level during ulcerative colitis.
Effect of *B. tomentosa* (10mg/kg b.wt.) and sulfasalazine (100mg/kg b.wt) on colonic tissue GSH level in rats with acetic acid-induced ulcerative colitis (mean of 6 animals±SD). As compared with acetic acid alone treated control group (one-way ANOVA followed by Dunnett’s t test), **p < 0.01.
Effect of *B. tomentosa* (10mg/kg b.w.) and sulfasalazine (100mg/kg b.wt) on colonic tissue SOD level in rats with acetic acid-induced ulcerative colitis (mean of 6 animals±SD). As compared with acetic acid alone treated control groups (one-way ANOVA followed by Dunnett’s t test), **p < 0.01.
Effect of *B. tomentosa* (10mg/kg b.w.) and sulfasalazine (100mg/kg b.wt) on colonic tissue GPX level in rats with acetic acid-induced ulcerative colitis (mean of 6 animals±SD). As compared with acetic acid alone treated control group (one-way ANOVA followed by Dunnett’s t test), **p < 0.01.
Treatment with *B. tomentosa* significantly inhibited acetic acid induced NO production and iNOS expression in colon. The serum nitric oxide level was also found to be decreased to 46.74 µM after *B. tomentosa* treatment compared with colitis control group (78.08 µM) (Fig 3.6). The iNOS expression studies showed significant inhibition after *B. tomentosa* treatment in a dose dependent manner (Fig 3.7).

### 3.3.5 Effect of *B. tomentosa* on colon Tumor necrosis factor-α (TNF-α), Myeloperoxidase (MPO) and Lactate dehydrogenase (LDH) level during ulcerative colitis

Effect of *B. tomentosa* on colon TNF-α, MPO and LDH level is shown in Fig 3.8, Fig 3.9 and Fig 3.10 respectively. The increased TNF-α production during ulcerative colitis (135.41 pg/mg tissue) was found to be decreased by the treatment with *B. tomentosa*. *B. tomentosa* at a concentration of 10mg/kg B.wt. could reduce the TNF-α level to 40.91 pg/mg tissue. Similarly the MPO and LDH level was also found to be decreased to 26.30 ng/g tissue and 787.67 U/L respectively after *B. tomentosa* treatment (10mg/kg B.wt). The MPO and LDH level in colitis control group was found to be 92.18 ng/g tissue and 2046.67 U/L respectively. The standard drug sulfasalazine could significantly reduce the TNF-α, MPO and LDH level to 42.47 pg/mg tissue, 27.17 ng/g tissue and 742.67 U/L respectively.
Fig 3.6: Effect of *B.tomentosa* on the serum NO level during ulcerative colitis.

Values are expressed as mean ± SD. *B.tomentosa* was administered once daily for 5 days before induction of colitis and 24 hours after fasting colitis was induced with 3% of acetic acid. **p < 0.01 compared with colitis control group.
Fig 3.7: Effect of *B. tomentosa* on the iNOS expression during ulcerative colitis.

Values are expressed as mean ± SD. *B. tomentosa* was administered once daily for 5 days before induction of colitis and 24 hours after fasting colitis was induced with 3% of acetic acid. Colon tissues were dissected out, washed with ice cold phosphate buffered saline (pH 7.4) and used for the estimation of iNOS expression studies.**p < 0.01 compared with colitis control group.
Fig 3.8: Effect of *B. tomentosa* on colon Tumor necrosis factor-α (TNF-α) during ulcerative colitis.

Values are expressed as mean ± SD. *B. tomentosa* was administered once daily for 5 days before induction of colitis and 24 hours after fasting colitis was induced with 3% of acetic acid. Colon tissues were dissected out, washed with ice cold phosphate buffered saline (pH 7.4) and used for the estimation of TNF-α. **p < 0.01 compared with colitis control group**
Fig 3.9: Effect of *B. tomentosa* on colon myeloperoxidase (MPO) during ulcerative colitis.

Values are expressed as mean ± SD. *B. tomentosa* was administered once daily for 5 days before induction of colitis and 24 hours after fasting colitis was induced with 3% of acetic acid. Colon tissues were dissected out, washed with ice cold phosphate buffered saline (pH 7.4) and used for the estimation of MPO. **p < 0.01** compared with colitis control group.
Fig 3.10: Effect of *B. tomentosa* on colon lactate dehydrogenase (LDH) during ulcerative colitis.

Values are expressed as mean ± SD. *B. tomentosa* was administered once daily for 5 days before induction of colitis and 24 hours after fasting colitis was induced with 3% of acetic acid. Colon tissues were dissected out, washed with ice cold phosphate buffered saline (pH 7.4) and used for the estimation of LDH. **p < 0.01 compared with colitis control group.
3.3.6 Effect of *B. tomentosa* on histological changes on colon

Effect of *B. tomentosa* on histological changes during colitis is shown in Fig 3.11. The normal colon mucosa (Fig 3.11A) showed no inflammation or necrosis. The colitis control animals showed ulceration of colonic mucosa, diffuse inflammatory cell infiltration in the mucosa. The crypts were found to be distorted and there was loss of epithelium (Fig 3.11B). Treatment with *B. tomentosa* (Fig 3.11C) and sulfasalazine (Fig 3.11D) showed marked reduction in the severity of cell damage.

3.3.7 Effect of *B. tomentosa* on expression of iNOS and COX-2 immunostaining

Colonic iNOS expression was primarily observed on neutrophils and smooth muscle cells with a parse distribution in the epithelial cells in naive controls (Figure 3.12). Immunohistochemical examination revealed that iNOS was unregulated in acetic acid-induced colitis group and was localized in the infiltrated inflammatory cells. At the same time treatment with *B. tomentosa* or sulfasalazine reduced the overexpression of iNOS induced by acetic acid treatment. Similarly, in normal groups, COX-2 expression was scarcely found in the surface epithelium and mononuclear cells of lamina and propria of mucosa. Compared with normal animals, the expression of COX-2 was found to be elevated in cells of surface epithelium and in cells of the inflammatory infiltrate in the acetic acid-induced ulcerative colitis group. Animals treated with *B. tomentosa* or sulfasalazine displayed lower level of COX-2 expression in acetic acid induced colitis group (Figure 3.13).
Fig 3.11: Effect of *B.tomentosa* on colon histology

A) Specimen from a normal rat showing colon with normal mucosa
B) Control specimen from acetic acid induced host not related with extract showing colitis with large necrotic destruction of epithelial cells, areas of hemorrhage, submucosal edema and inflammatory cellular infiltration
C) Colitis + *B. tomentosa* (10mg/kg.B.wt)  
D) Colitis + sulfasalazine (100mg/kg.B.wt.) (40x magnification).
Fig 3.12 Immunohistochemical localization of iNOS during ulcerative colitis

(A) Immunohistochemical localization of iNOS in normal control, (B) Positively stained brown granules for iNOS were significantly increased in both number and intensity in colonic tissue of acetic acid treated rats. (C & D) sulfasalazine (100 mg/kg.b.wt) and Bauhinia tomentosa (10 mg/kg.b.wt) treated reduced colonic iNOS expression of acetic acid treated rats (40x magnification).
Fig 3. Immunohistochemical localization of COX-2 during ulcerative colitis

(A) Immunohistochemical localization of COX-2 in normal control, (B) Positively stained brown granules for COX-2 were significantly increased in both number and intensity in colonic tissue of acetic acid treated rats. (C & D) sulfasalazine (100 mg/kg.b.wt) and Bauhinia tomentosa (10 mg/kg.b.wt) treated reduced colonic COX-2 expression of acetic acid treated rats (40x magnification).
3.4 Discussion

Inflammatory bowel diseases (IBD), including Crohn’s disease and Ulcerative colitis, are life-long and recurrent disorders of the gastrointestinal tract with unknown etiology. Although ulcerative colitis etiology is largely unknown literature suggests that multiple immune, genetic and environmental factors influence both the initiation and progression of colitis (Martin et al., 2006). Ulcerative colitis has been shown to increase the risk of colorectal cancer (Talero et al., 2008). There is evidence for an intense local immune response associated with infiltration of lymphocytes and macrophages into the mucosa followed by release of reactive oxygen species, cytokines and other inflammatory mediators resulting in tissue damage, which recruits additional inflammatory cells to the site of repair (Talero et al., 2008). The increase in NO during colitis leads to an increased production of peroxynitrite which ultimately results in oxidation of lipids and proteins, DNA strand breaks, depletion of adenosine triphosphate and tissue damage (Sunkara et al., 2011) Antioxidants such as SOD, GPx and GSH impart protection against oxidative damage produced by reactive oxygen species and nitrogen species (Danese 2010). In the present study, the effect *B.tomentosa* against acetic acid-induced colitis was examined.

The decrease in colon wet weight can be directly correlated with the degree of inflammation (Rachmilewitz et al., 1989). The present study showed that treatment with *B.tomentosa* could reduce the colon wet weight and macroscopic lesion score compared with colitis control group. Recently, many evidences have indicated that a common link between inflammatory bowel disease may be related with oxidative stress (Rachmilewitz et al., 1989). The free radicals produced during oxidative damage may lead to biological membrane lipid peroxidation, resulting in severe cell damage and play a significant role in the pathogenesis of disease. Therefore receiving free radical scavengers and reducing oxidative stress may benefit for reducing ulcerative colitis (Jena et al., 2012). In this study, colitis control animals exhibited
increased levels of LPO in colon tissue. *B. tomentosa* treatment significantly reduced the increase of LPO levels. The results could explain the inhibited activity of ulcerative colitis by *B. tomentosa* treatment may be related, partially at least, to the antioxidant and free radical scavenging ability. GSH is an important intracellular defense antioxidant agent in mammalian gut. GSH is involved in the repair mechanism, inhibits free radical damage, and enhances the antioxidant activity of Vitamin C. Intracellular GSH is the main reducing agent for colonic epithelial cells which neutralize hydrogen peroxide. During inflammation, GSH level depletes resulting in severe degradation of jejunum and colon mucosa. Depletion of GSH may result in increased MDA, an end product of lipid peroxidation which ultimately results in cellular damage. Therefore, GSH plays a vital role in protecting the intestinal cells and as a defense mechanism against inflammation (Chavan et al., 2005). Treatment with *B. tomentosa* significantly increased the colonic GSH levels, preventing the manifestation of mucosal injury during inflammatory bowel disease. Superoxide dismutase (SOD) is a key enzyme which inactivates superoxide ion by transforming it into more stable metabolite, hydrogen peroxide. This H$_2$O$_2$ is further converted to water by catalase or GPx. The activities of these enzymes usually are balanced which maintains a steady state of ROS. The SOD level was found to be decreased during ulcerative colitis. Treatment with *B. tomentosa* significantly increased the colon SOD level in a dose dependent manner. This increased level could prevent the adhesion of circulating leucocytes to intestinal endothelium by blocking the expression of adhesion molecules such as vascular cell adhesion molecule and intracellular adhesion molecule and this needs further investigation. GPx is an antioxidant enzyme that helps in the scavenging and inactivation of free radicals thereby protecting the body against oxidative stress. GPx catalyze the reduction of H$_2$O$_2$ and a wide variety of organic peroxides (R-OOH) to the corresponding stable alcohols (R-OH) and water using cellular GSH as the reducing agent. There are reports which shows that the cytosolic GPx activity in rat colon tissue is altered in response to oxidative stress with associated disturbances in prostaglandin
synthesis that are modulated by anti-inflammatory salicylates (Drew et al., 2005). The present study shows that *B. tomentosa* could significantly decrease the colon GPx, thereby preventing oxidant mediated colon tissue damage.

Myeloperoxidase (MPO) is an enzyme most abundantly secreted in neutrophils and stored in azurophilic granules of the neutrophils. It is also secreted at lower concentration in monocytes and macrophages (Klebanoiff 2005). The level of MPO activity is directly proportional to tissue neutrophil content, thereby it produces more hypochlorous acid which is cytotoxic (Mullane et al., 1985; Higa et al., 1997). The present study showed that oxidative damage associated with induction of acetic acid resulted in an increased in MPO activity, as reported previously (Galvez et al., 2000; Paiva et al., 2002; Islam et al., 2008). A significant reduction in MPO by treatment with *B. tomentosa* extract exhibits the intestinal anti-inflammatory effect in the experimental colitis model.

Nitric oxide (NO) is an important proinflammatory mediator that is largely produced by three isoforms of NOS: neuronal NOS (NOS1), inducible NOS (iNOS or NOS2) and endothelial NOS (NOS3) (Lih-brody et al., 1996). The nitric oxide and iNOS has been reported as potential mediators for colitis (Rachmilewitz et al., 1995; Guslandi 1998). However the exact role of NO in intestinal inflammation is unknown. The uncontrolled inflammatory response and oxidative stress could induce dramatic changes in colon tissue morphology and intestinal microvascular function during colitis. The most noticeable changes associated with colitis are enhanced interstitial edema, increased arteriolar blood flow, fluid exudation across intestinal capillaries, thickening of the intestinal wall, and colon inflammation, all of which are associated with inflammatory mediators such as NO (Gillberg et al., 2012). The over production of NO through iNOS upregulation by the intestinal epithelium has a direct role in the regulation of disease-specific, NO-related genes that are involved in NO-related inflammation during ulcerative colitis (Kolios et al., 2004). The present study showed that administration of *B. tomentosa*
significantly inhibited the NO production and iNOS expression, which ultimately results in the prevention of peroxynitrite formation from inflammatory cells such as macrophages, neutrophils and endothelial cells, which counter inflammation during ulcerative colitis.

Ulcerative colitis has been associated with an intense local immune response which is associated with the recruitment of lymphocytes and macrophages followed by release of soluble cytokines and inflammatory mediators (Ogata et al., 2003). TNF-α, an important proinflammatory cytokine released from the macrophages and lymphocytes in the early inflammatory response plays an important role to activation and nuclear translocation of NF-kB, a heterodimeric transcription factor that translocates to the nucleus and mediates the transcription of a vast array of proteins involved in cell survival and proliferation as well as inflammatory response. TNF-α has been reported to play an integral role in the pathogenesis of inflammatory bowel disease (Sands and Kaplan 2007). Blocking TNF-α has been shown to inhibit colitis and colorectal cancer in animal models (Popivanova et al., 2008). Treatment with B.tomentosa significantly inhibited the TNF-α production in a dose dependent manner.

B.tomentosa has been reported to possess phytochemical constituents such as Kaempferol-3-O-rhamnoside, Kaempferol-3-O-rutinoside, Quercerin 3-O-glucoside and Quercerin 3-o-rutinoside (rutin). Kaempferol-3-O-rhamnoside, one of the active components present in Bauhinia tomentosa has been shown to inhibit cancer cell proliferation and promote apoptosis by the activation of the caspase signaling cascade (Diantini et al., 2012). Another compound quercerin 3-o-rutinoside commonly known as rutin has been shown to exhibit antitumour activity by targeting mitochondrial apoptotic pathway (Samanta et al., 2010). Rutin has been reported to inhibit B16F-10 induced experimental lung metastasis in C57BL/6 animals (Menon et al., 1995) and could reduce NF-kappa B, TNF-α and caspase-3 expression during renal inflammation (Arjumand et al., 1995). Kwon et al., (2005) had shown that rutin
could be useful for the prevention and treatment of inflammatory bowel disease and colorectal carcinogenesis through the attenuation of proinflammatory cytokine production. Further rutin (Quercetin 3-O-rutinoside) has been reported to inhibit experimental colitis by inhibiting TNF-α dependent NF kappa B activation (Kim et al., 2005).