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Original Article

Protective effect of *Lagerstroemia speciosa* against dextran sulfate sodium induced ulcerative colitis in C57BL/6 mice

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Abstract: The protective effect of methanolic extract of Lagerstroemia speciosaleaves (LS) was evaluated against dextran sulfate sodium (DSS) induced ulcerative colitis in C57BL/6 mice. The administration of DSS (2.5% in drinking water ad libitum) in C57BL/6 mice induced ulcerative colitis in 7 days. The LS was orally administered for 7 days at daily doses of 100 and 200 mg/kg. At the end of 7 days of treatment the animals were sacrificed, colonic tissues were removed and processed for further analysis of oxidative stress, and histopathology. In DSS treated mice the oxidative stress markers were elevated compared to controls. There was also significant reduction in the anti-oxidant defense levels marked by reduced cellular glutathione, catalase, and superoxide dismutase. The DSS-induced damage to the colon epithelium was evident from a significant increase in the lipid peroxidation. The histology of colon sections revealed inflammatory changes and marked impairment in the integrity of the mucosal lining with inflammatory changes. Both the doses of LS significantly prevented DSS-induced inflammatory and ulcerative damages of the colon, reduced lipid peroxidation and also restored the levels of innate antioxidants in the colon tissue. These findings indicate the protective effects of LS against the DSS-induced inflammatory and oxidative damage in the mouse colon. Further investigation involving bioactivity guided fractionation of the LS can yield potent constituent which may have a significant role in the treatment of inflammatory bowel disease and ulcerative colitis.

Keywords: DSS, ulcerative colitis, oxidative stress, lagerstroemia, 5-amino salicylic acid

Introduction

Ulcerative colitis (UC) and Crohn's disease (CD) are multifactorial inflammatorybowel diseases (IBDs). The occurrence of IBDs is progressively increasing around the globe [1]. The pathogenesis of IBDs involves immune-inflammatory mechanisms [2]. The CD can affect any part of the GIT; resulting in the formation of the narrowed, thickened and fibrotic bowel, in severe cases the focal inflammation promotes the stellate ulceration in the bowel. While UC, being a mucosal disease can spread to entire colon or restrained up to a part of the colon, evoking in erythmatous to haemorrhagic, granular mucosa with certain extensions to terminal colon forming the backwash ileitis [3, 4]. UC is characterized by an irregular damage to the intestinal mucosa resulting from multiple etiologies comprising of genetic, environmental, microbial and immune inflammatory mediators [5, 6]. The most disturbing symptoms and consequences of UC are ulceration, loss of blood through fecal matter, abdominal pain, diarrhoea, loss of body weight, loss of minerals from the bones and osteoporosis [7, 8]. The IBDs evoke increased energy expenditure and nutritional deficiencies like malnutrition, malabsorption, disturbed digestion, loss of gastrointestinal proteins [9]. The treatment goals for IBDs include prevention and treatment of complications and restoration of nutritional deficits to improve patient's quality of life. The anti-inflammatory drugs include immunosuppres-

Table 1. Calculation of disease activity index score

Score	Weight loss	Stool Consistency	Bleeding
0	Normal	Normal (well-formed pellets)	Not observed
1	1-5%	Normal	Not observed
2	6-10%	Loose (pasty stools that do not stick to the anus)	Occult
3	11-15%	Loose (pasty stools that do not stick to the anus)	Occult
4	> 15%	Diarrhea (Liquid stools that stick to the anus)	Gross bleeding

sants, biologic agents, antibiotics, and drugs to suppress inflammatory responses and to relieving symptoms are used in treatment of IBDs [10]. The Complementary and alternative therapies are also considered to be effective in treatment of IBDs however, there is a need for well-planned preclinical and clinical investigations to supports such claims [11]. In present investigation, the efficacy of methanolic extract of *Lagerstroemia speciosa* leaves (LS) in reducing the dextran sodium sulphate (DSS)-induced ulcerative colitis in mice is substantiated.

The *Lagerstroemia speciosa* leaves well known as Banaba leaves are reported to possess multiple biological activities including anti-inflammatory [12, 13], antinociceptive [14], anti-obesity, anti-cholesterolemic [15], hypoglycaemic [16], antidiabetic [17], organ protective and, antioxidantactivities [18]. Majority of the studies have concluded that the active constituent of LS is corosolic acid [19]. Other phytoconstituents of LS including gallotanins and lagerstroemin may also contribute to the bioactivities of LS [20]. The efficacy of LS in IBDs still remains unexplored and there is a dearth of reports on such efficacy of isolated constituents of banaba leaves in the IBDs. Hence, in present preliminary study, the efficacy of LS is investigated in the experimental model of DSS-induced UC.

Materials and methods

Drugs and chemicals

Dextran sodium sulfate (CAT no. 160110) was purchased from MP Biomedical (Aurora, OH), amino salicylic acid (ASA) was purchased from Sigma Aldrich, USA. Hydroxylamine, 5-5-dithio bi's-(2-nitrobenzoic acid) (DTNB), thiobarbituric acid, triton X-100 and other chemicals for oxidative parameters measurement were purchased from Sigma Aldrich, USA. All other chemicals were of analytical grade.

Animals

The experimental protocols related to present study were approved by the Institutional Animal Ethical Committee of R. C. Patel Institute of Pharmaceutical Education and Research, Shirpur, India (Approval Number: RCPIPER/IAEC/15/02). The experiments were carried out according to the guidelines prescribed by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

Male C57BL/6 mice weighing 20-30 g were obtained from the animal house facility of Mahaveera Enterprises, Hyderabad (Andhra Pradesh), India. They were housed in controlled atmosphere at 22±2°C temperature, 65% relative humidity and 12 hr light-dark cycle. The animals had free access to standard food pellets (Amrut Feed, Pune, India) and drinking water; the acclimatization period was of one week.

Experimental protocol

The mice were randomly allocated to 5 treatment groups containing 6 mice each. The naïve control and DSS-control groups were orally administered with 0.5 ml of carboxymethyl cellulose solution (0.5% CMC solution) everyday for 7 days. All groups except the control group received 2.5% w/v DSS solution instead of the drinking water. The ASA-treated group was administered with 100 mg/kg ASA suspended in 0.5 ml CMC solution. The mice in LS low dose and LS high dose groups were orally administered daily with 100 and 200 mg/kg of LS as a suspension in 0.5 ml CMC. We maintained the record of daily consumption of DSS solution of the exposed study groupsto confirm a uniform exposure of individual mouse to DSS.

Daily record of observational parameter

The body weight was monitored every day in between 9.00 am to 10.00 am. Loss of body weight was calculated as the difference between the initial and final weight. The stool consistency and rectal bleeding were scored according to the grading scheme mentioned in **Table 1**. We estimated the daily food intake per treatment group housed in separated cage by

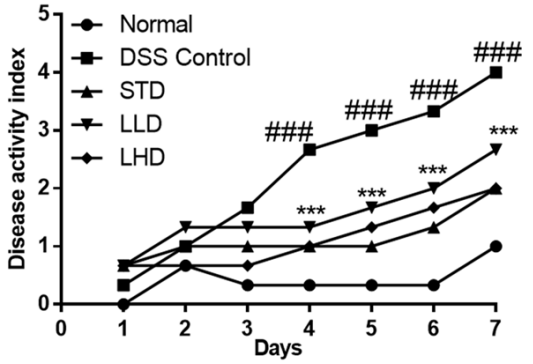

Figure 1. Effect of LS on disease activity index in DSS induced colitis. Data was expressed as mean ± SEM (n=6). Statistical significances were determined using two way analysis of variance (ANOVA) followed by Bonferroni's post hoc test. ###P<0.001 as compared to normal, ***P<0.001 as compared to DSS control. DSS: Dextran sulphate sodium, STD: Standard drug 5-Amino salicylic acid, LLD: Low dose of lagerstroemia (100 mg/kg), LHD: High dose of lagerstroemia (200 mg/kg).

determining the remnant of 40 g of food pellets provided on the previous day.

Assessment of colitis severity

Severity of colitis was assessed daily using a Disease Activity Index (DAI) scoring system accounting for body weight loss, stool consistency, and rectal bleeding as described previously [21]. Following formula was used for determination of DAI-DAI = Score of weight loss + Stool consistency + Bleeding/3.

Measurement of colon length

The mice were sacrificed at the end of treatment schedule on day 7. Each mouse was dissected to isolate the colon. Colon was separated from the proximal rectum close to its passage under the pelvisternum. The colon length was measured by placing it on a graph paper with standard measured length in centimeters. The results were documented as photographsa part of colon tissue was immediate transferred to ice-cold phosphate buffered saline (pH7.4) and used for estimation of the oxidative stress parameters. Remaining of the colon was fixed in buffered 10% formalin solution for histological investigations.

Estimation of the DSS-induced oxidative stress in the colon homogenates

The colon tissue was minced and homogenized in ice-cold Tris hydrochloride buffer (10 mM, pH7.4). The homogenate so formed was centrifuged at 7000 rpm for 20 min using high-speed centrifuge, and the supernatant was used for estimation of oxidative stress parameters.

Estimation of reduced glutathione (GSH)

GSH was determined by 5-5-dithio bis-(2-nitrobenzoic acid) (DTNB) method. It involved the treatment of the colon homogenate with DTNB solution and determination of the absorbance of the developed yellow color at 412 nm [22].

Estimation of lipid peroxidation (LPO)

The colon homogenate samples were treated with 3 ml of 1% phosphoric acid solution and 1 ml of 0.6% thiobarbituric acid aqueous solution. The reaction mixture was heated at 80°C for 45 min, cooled in an ice bath extracted with 4 ml of n-butanol. The n-butanol Fraction was separated and its optical density was measured at 532 nm. Theoptical density was used to determine the thiobarbituric acid reactive substances (TBARs) that represented the malondialdehyde contents in the colon homogenate [23].

Estimation of catalase

To estimate the activity of catalase, we added 50 µl of the colon homogenate to 1 ml of 10 mM H_2O_2 solution at room temperature and determined the optical density of the mixture at an interval of 60 seconds for 180 seconds. The decrease in optical density was use determined [24].

Estimation of superoxide dismutase (SOD)

SOD was estimated by nitro blue tetrazolium (NBT) dye reduction assay as described previously [25]. The colon homogenate was centrifuged at 15000 rpm for 15 minute at 4°C and the supernatant was used for estimation of SOD activity. The rate of reduction of NBT triton in presence of Na_2CO_3, hydroxylamine and 0.3% Triton X was spectrophotometrically recorded at 560 nm. The rate of reduction of NBT was used for calculation of the SOD activity.

Histopathological evaluation

The colons samples fixed in 10% buffered formalin were paraffin-embedded, sectioned at 5 µm thickness, and stained with hematoxylin and eosin stain. Histological evaluation of was performed in a blinded manner by a skilled

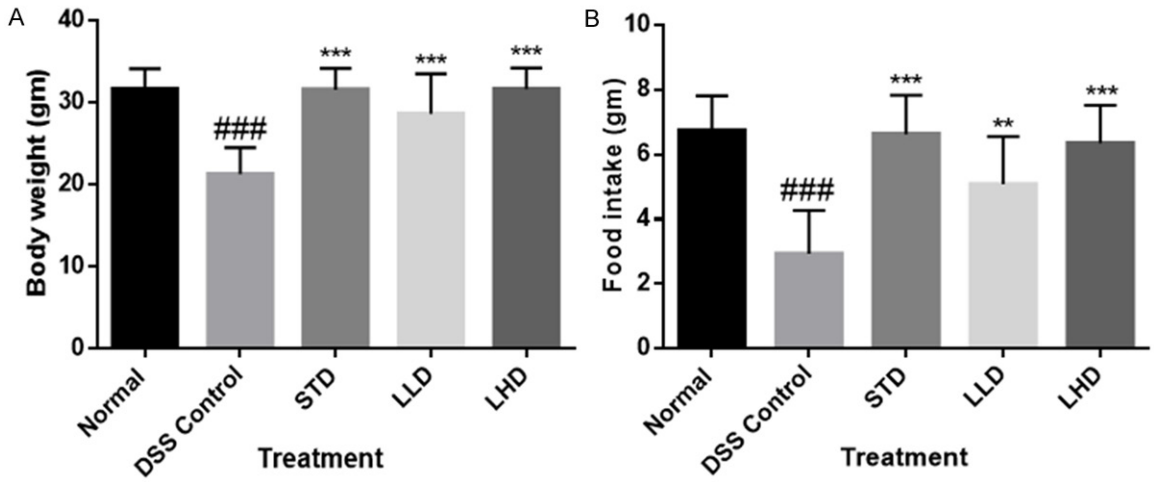

Figure 2. Effect of LS on body weigh changes and food intake in DSS induced colitis. Data was expressed as mean ± SEM (n=6). Statistical significances were determined using one way analysis of variance (ANOVA) followed by Dunnette's post hoc test. ###P<0.001 as compared to normal, **P<0.01, ***P<0.001 as compared to DSS control. DSS: Dextran sulphate sodium, STD: Standard drug 5-Amino salicylic acid, LLD: Low dose of lagerstroemia (100 mg/kg), LHD: High dose of lagerstroemia (200 mg/kg).

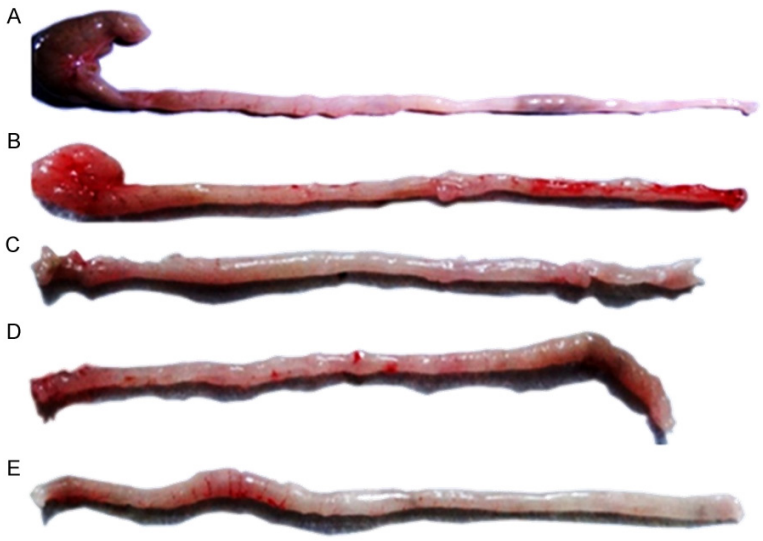

Figure 3. Effect of LS on colon length in DSS induced colitis. A: Normal; B: DSS control; C: STD; D: LLD; E: LHD. DSS: Dextran sulphate sodium, STD: Standard drug 5-Amino salicylic acid, LLD: Low dose of lagerstroemia (100 mg/kg), LHD: High dose of lagerstroemia (200 mg/kg).

pathologist. The grading index was as follows: inflammation severity (0: none, 1: mild, 2: moderate, 3: severe); extent of inflammation (0: none; 1: mucosa, 2: mucosa and submucosa, 3: transmural); and crypt damage (0: none, 1: basal one-third damaged, 2: basal two-thirds damaged, 3: only surface epithelium intact, and 4: entire crypt and epithelium lost) [24].

Statistical analysis

The statistical analysis was performed using Graph Pad Prism version 6.0 software, USA. Data were expressed as means ± standard error mean for each group. Statistical analysis was performed using the analysis of variance (ANOVA) followed by Bonferroni's post hoc test or Dunnette's post hoc test with comparison of all column pairs. A value of $*P<0.05$ was considered statistically significant.

Results

The preliminary analysis of phytoconstituents

In the preliminary qualitative chemical analysis of the SL we identified the presence of triterpenes, tannins, ellagic acids, glycosides and flavones (Data was not shown).

LS treatment reduced the disease activity index in DSS treated mice

The DAI score of DSS treated mice was significantly higher that the only vehicle treated control group mice. From the 4th day onwards the

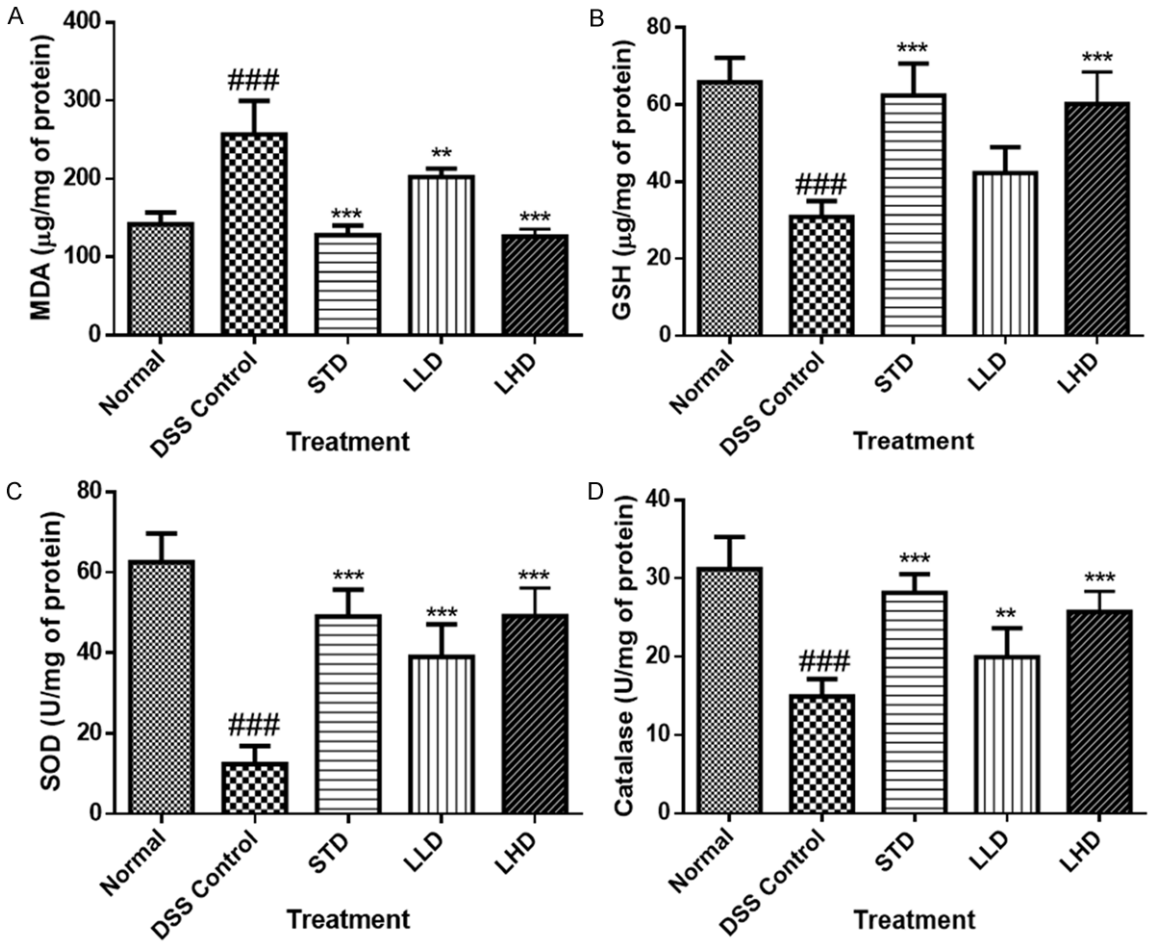

Figure 4. Effect of LS on oxidative stress in DSS induced colitis. A: Malondialdehyde content; B: Glutathione reductase; C: Superoxide dismutase; D: Catalase. Data was expressed as mean ± SEM (n=4). Statistical significances were determined using one way analysis of variance (ANOVA) followed by Dunnette's post hoc test. ###P<0.001 as compared to normal, **P<0.01, ***P<0.001 as compared to DSS control. DSS: Dextran sulphate sodium, STD: Standard drug 5-Amino salicylic acid, LLD: Low dose of lagerstroemia (100 mg/kg), LHD: High dose of lagerstroemia (200 mg/kg).

DSS receiving mice suffered from moderate bleeding along with loosed stools. These characteristic DSS-induced alterations in the stool consistency and bleeding were absent in the low and high dose LS treated mice (**Figure 1**).

LS prevented DSS-induced body weight loss

From third day onwards a significant decrease in the body weights was observed in the DSS treated mice. On the last day of treatment DSS treated group had average body weight of 16.75±1.8 g as compared to the naïve control group in which average weight was 30±1.8 g. The low and high dose LS treated groups had averaged body weights of 24.4±0.24 g and 24.4±1.4 g respectively. The standard drug treated group weighed 26.5±1.2 g (**Figure 2A**). The food intake on the last day of treatment is shown in **Figure 2B**. DSS administration significantly lowered the food intake as compared to the control group. Both low and high dose LS treated groups had average food consumption significantly more than only DSS treated group. This effect of LS was similar to that of ASA (**Figure 2B**).

LS reversed the DSS-induced damage to colon

DSS administration damaged the structure of colon and induced ulcers, hemorrhagic lesions, and shortened the colon. In the DSS treated control group the average colon length was 6.84±0.13 cm as compared to 10.3±0.27 cm observed in the control group. Average colon lengths of 7.9±0.49 cm and 9.1±0.48 cm were

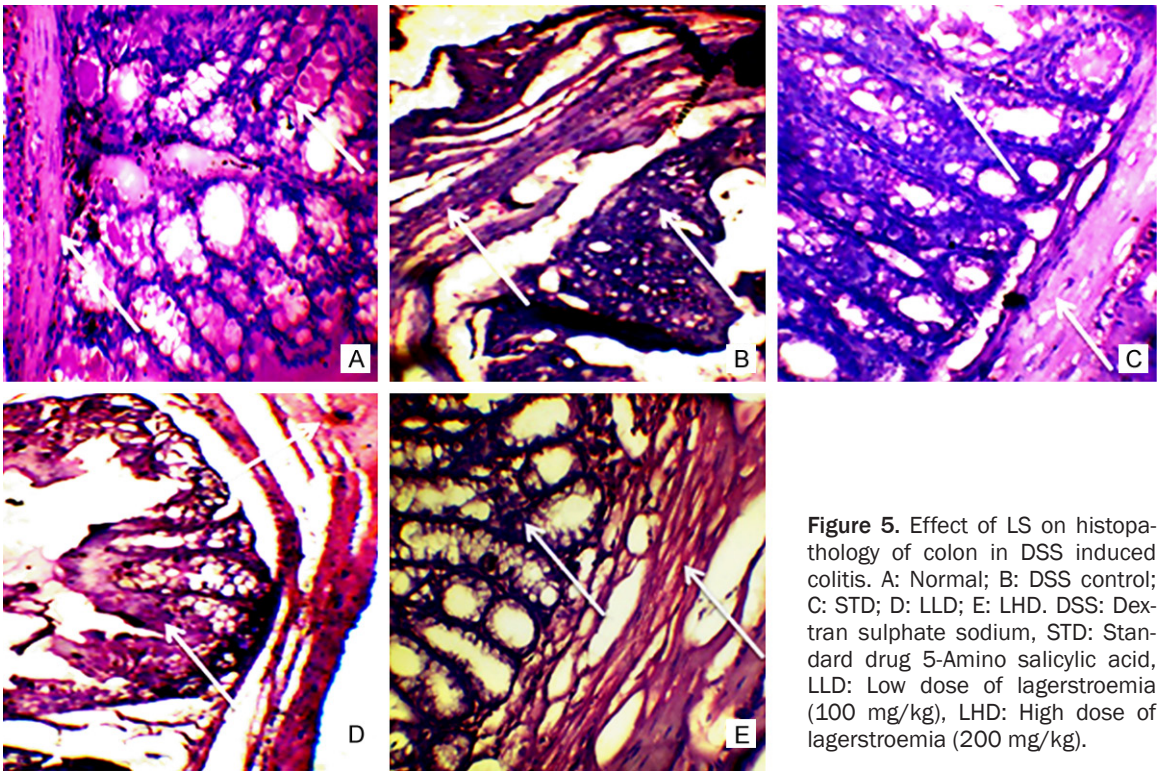

Figure 5. Effect of LS on histopathology of colon in DSS induced colitis. A: Normal; B: DSS control; C: STD; D: LLD; E: LHD. DSS: Dextran sulphate sodium, STD: Standard drug 5-Amino salicylic acid, LLD: Low dose of lagerstroemia (100 mg/kg), LHD: High dose of lagerstroemia (200 mg/kg).

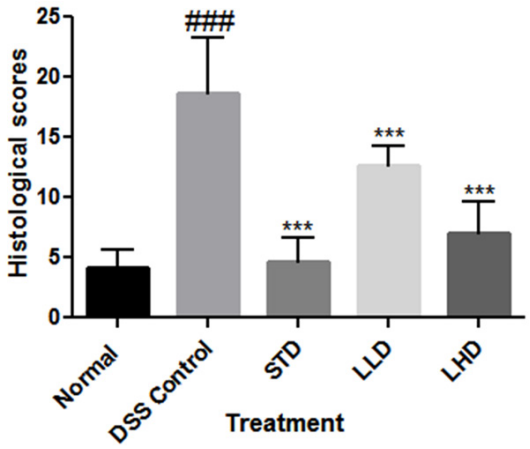

Figure 6. Histological scoring in DSS induced colitis. Data was expressed as mean ± SEM (n=6). Statistical significances were determined using one way analysis of variance (ANOVA) followed by Dunnette's post hoc test. ###P<0.001 as compared to normal, ***P<0.001 as compared to DSS control. DSS: Dextran sulphate sodium, STD: Standard drug 5-Amino salicylic acid, LLD: Low dose of lagerstroemia (100 mg/kg), LHD: High dose of lagerstroemia (200 mg/kg).

observed in the low and high dose LS treated groups. The colon length was 8.68±0.23 cm in the ASA treated mice (**Figure 3**).

LS treatment protected mice colon from DSS-induced oxidative stress

DSS treatment significantly reduced the activities of SOD and catalase and decreased the levels of GSH in the colon tissue (P<0.001). In congruence with these alterations, it induced the lipid peroxidation in colon tissue which was evident from a significant increase in the levels of TBARs. LS counteracted these deleterious effects of DSS (**Figure 4**).

The histological architecture of colon was protected by LS treatment

Figure 5 highlights the histological alterations in colon samples of mice which were exposed to different treatments. The **Figure 5A** mice colon showed normal structure and architecture, in **Figure 5B** light micrograph of DSS-control group colon showing destructed epithelial architecture with loss of crypts, epithelial integrity, sub mucosal oedema, intense inflammatory cellular infiltration, focal confluent necrosis of muscle fiber with fibroblastic proliferation and phagocytosis along with extravasations of red blood cells, in **Figure 5C** mice treated with standard drug ASA (100 mg/kg/day) showing intact epithelial cell with no oedema,

inflammation and necrosis, in **Figure 5D** LLD (100 mg/kg/day) treated mice colon showing mild congestion in epithelial cells, in **Figure 5E** mice treated with LHD (200 mg/kg/day) showing marked restoration of normal architecture. The histological findings were graded as described above. The comparison of the scores is represented as a bar chart in **Figure 6**.

Discussion

Ulcerative colitis is an idiopathic chronic relapsing-remitting inflammatorydisorder characterized by diarrhoea and rectal bleeding that affects colon [26]. The DSS-induced acute colitis in mice serves as a validated model to investigate the pharmacodynamics of drugs affecting colitis [27]. This model features clinical, biochemical, and histological characteristics of human ulcerative colitis and presents predictable disease progression [28]. In the ulcerative colitis, the intestinal lamina propria is associated with macrophages and lymphocytes mononuclear cell infiltration. Macrophages, a major population of tissue-resident mononuclear phagocytes, play a key role in recognition and elimination of bacteria. They produce proinflammatory cytokines in response to activated Th1 cells and induce tissue damage [29]. The DSS-induced UC model in mice is a preferred model due to its rapidity, simplicity, reproducibility and controllability [30].

In present study we estimated the disease activity index using the score of stool consistency, blood loss and loss in body weight. This scale has been validated previously [31]. DSS induced increase in the DAI was evident in this study. Characteristic Colon length is a definite indicator of ulcerative colitis. The total decrease in the colon length in for DSS fed mice was another indicator of the colon damage induced by DSS. Similar findings are reported previously [32]. The gross pathological alterations including hemorrhagic and ulcerative lesions were evident in the DSS administered group. These observations indicate the exposure of lamina propria and sub mucosa to chemical injury by DSS. In our study, we observed that DSS treated mice having shortened the length of colon and increase the inflammation, while entire pre-treated groups showed protective effect on colon length and inflammation. During intestinal inflammation, there is an intense flux of circulating neutrophils into the inflamed mucosa, which amplifies the inflammation by releasing large amounts of superoxide and hydrogen peroxide and thereby increasing the oxidative stress [33]. These effects of DSS were significantly reduced by LS. The antioxidative properties of LS against chemically induced organ damages are extensively reported. The LS exerted protection of colon tissues against DSS-induced UC can be attributed to the antioxidant activity of LS [34].

Oxidative stress is one of the most decisive factors inductions of UC which damages cellular macromolecules such as DNA, lipids, and proteins. In general, ROS are known to neutralize the endogenous antioxidant enzymes. SOD converts O_2 to H_2O_2, which is subsequently neutralized by catalase activity [35]. MDA, a by-product of lipid peroxidation increases during the induction of the UC. We noted a significant rise in the levels of MDA in colon homogenates of the DSS-treated group. LS administration with DSS limited the elevations in MDA levels. This effect further confirms the ability of LS to inhibit the DSS induced oxidative damage of the colon. LS is also known to increase the activity of SOD and other antioxidant enzymes. In the present study, administration of LS significantly increased the activity of the innate antioxidant enzymes. These findings indicate that LS protects the colon from DSS induced damage through its potent antioxidative effects. The LS exerted strong antioxidant effect in DSS-induced colitis in mice.

The sulfate group confers negative charge to the DSS molecules which makes it toxic to the colonic epithelium inducing its erosion. This ultimately damages the barrier integrity of epithelium resulting in to an increased epithelial permeability. The anticoagulant property of DSS aggravates intestinal bleeding. The reasons for distal colon specific and confined damages induced by DSS are unknown. This region of colon has dense bacterial flora however definite correlations remain to be established between the confined effects of DSS and the bacterial colonies. The mechanism by which DSS passes through mucosal epithelial cells remains unclear [36]. The specificity of DSS to the colon could be a function of water and electrolyte absorption in the presence of numerous bacteria.

Typical histological changes induced by DSS include mucin and goblet cell depletion, epithelial erosion and ulceration. DSS induces an influx of neutrophils, monocytes and, macrophages into the lamina propria and submucosa. Inflammatory alternations induce disarray and a widening of the gap between the base of the epithelium and muscularis along with deep mucosal lymphocytosis. All such typical changes were observed in the only DSS receiving mice in the present study. The treatment with LS protected the colon against the deleterious effects of DSS which was evident from a decrease in influx of neutrophils into the lamina propria and submucosa.

In conclusion, the present findings suggest that LS is an effective inhibitor of DSS-induced colitis in mice. The administration of LS to mice treated with DSS attenuated the acute inflammation in colon. Therefore, LS may reverse the deleterious state induced by DSS. Further investigation on the active constituents of LS may yield therapeutically usable molecules which can have a role in the treatment of IBDs.

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Disclosure of conflict of interest

None.

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