Protective Effect of Spirulina Fusiform in Chronic Colitis in Male Albino Rats

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Abstract:

Background/Objectives: In this work, a modified low dose model of induction of ulcerative colitis was used including the administration of single intra-rectal instillation of 1 ml of 5% acetic acid followed by 2% dextran sodium sulphate for 7 days. Moreover, aqueous suspension of Spirulina (500 mg /kg stomach gavage) was prophylactically used 7 days before disease induction. Antioxidant, anti-inflammatory effect was achieved by spirulina. Aim: The aim of this study was to determine the effectiveness of spirulina as a new, safe in treatment of ulcerative colitis. Material and methods: Chronic UC was induced by 1ml 5% intracolonic acetic acid instillation in adult male albino rats as single dose following by adding 2% DSS orally in drinking water for 7 days. Intragastric administration of spirulina. (500 mg/kg/day) was administrated 7 days before acetic acid instillation and continued for the end of the experiment. Disease activity index (DAI), colonic oxidative stress markers, tumour necrosis factor (TNF)-a levels, IL1B, and the colonic histopathological changes were observed. Results: spirulina attenuated the severity of the acetic acid -DSS induced colitis through improving the DAI, the colonic oxidative stress markers, TNF-a, IL1b, morphological and partially ameliorate all histopathological changes. Conclusion: Therapeutically, spirulina could be administered in chronic colitis as potent antioxidant compounds and anti-inflammatory effect of phyocyanin will become a therapeutic strategy of choice for UC to improve the quality of life if sufficient clinical trials are available.

Keywords: spirulina, oxidative stress, tumour necrosis factor.
Introduction:

Inflammatory bowel disease (IBD), including ulcerative colitis (UC), is characterized by chronic relapsing intestinal inflammation. It has been a worldwide health-care problem with a continually increasing incidence among all ages [1].

Although the complexed pathophysiological events in IBD, the multifaceted interactions between the genetic/environmental/microbial factors and the triggered oxidative stress, and pro-/inflammatory burdens are emphasized, which is under the tight innate and adaptive immune regulations [2].

Most of rat models of ulcerative colitis use immune-active substances e.g. dextran sodium sulphate or carrageenan or irritants such as acetic acid as single inducing agents. This approach copes well with clinical picture but less coping with pathogenesis [3]. In this study traditional dextran sodium sulphate rat model is modified by prior administration of single intracolonic acetic acid instillation to cope with the recent advances in pathogenesis [4] which emphasized breaking of mucosal barrier as a triggering event, in later immunologically mediated chronic colitis. Moreover, the dose and duration of dextran sodium sulphate was reduced.

Currently, available therapies for IBD are only effective in ameliorating the disease symptoms while having many concomitant disadvantages [5]. In this context, a number of recent studies have renewed interest in the antioxidant potential of Spirulina fusiform for the management of inflammatory conditions and oxidative damages [6]. This algae has been used as a source of protein and vitamin supplement in humans without any significant side-effects. Apart from its high (up to 70%) content of protein, it also contains vitamins, especially B12 and provitamin A (b-carotenes), and minerals, especially iron. It is also rich in phenolic acids, tocopherols and g-linolenic acid (7). C-phycocyanin (C-PC), one of the major biliproteins of SP, is reported to exhibit antioxidant, radical scavenging properties, as well as selective cyclooxygenase-2 inhibition, anti-inflammatory and anticancer effects [8].

The aim of this study is to determine the therapeutic effectiveness of spirulina fusiform in acetic acid-DSS induced chronic colitis in rats.
Materials and methods:

Animals

Twenty-four adult male albino rats, of 120–150 g, were used in this study. All rats were housed under normal light/dark cycle, at temperature of 25 °C, housed in plastic polyethylene cages (eight per cage) with free access to food and water, being maintained on a diet composed of (20% casein, 15% corn oil, 55% corn starch, 5% salt mixture, and 5% vitamins). Animals allowed for acclimatization for one week before the start of the study in the Pharmacology Department, Benha Faculty of Medicine. All experimental dealings were permitted by the institutional animal care and use committee, which is following the National Institutes of Health guide for the care and use of laboratory animals (Maryland, USA).

Drugs and chemicals

The DSS (Affymetrix, USA) was purchased as a white crystalline powder (MW 500000 Da). Spirulina purchased from (Sigma-Aldrich Company, St. Louis., USA).

Kits for malondialdehyde (MDA), superoxide dismutase (SOD), and catalase (CAT) were purchased from Biodiagnostic Company (Giza, Egypt), while TNF-a enzyme-linked immunoassay (ELISA) and IL1B purchased from (Sigma-Aldrich Company, St. Louis., USA). Faeces occult blood was determined using the occult blood test device (ACON Laboratories, Cairo, Egypt). Acetic acid was obtained from El-Nasr Chemical Co. (Cairo, Egypt). Hematoxylin and eosin: (E.Merck, Darmstadt, Indiana, USA).

Methods

Type of the study: experimental study, rats were divided randomly into 3 groups of 8 animals each.

Group 1: control normal group; rats without colitis, where no medications were allowed rectal instillation of saline on day 1 followed by given oral saline for 7 days

Group 2: None treated chronic colitis group. Chronic colitis was induced by intrarectal administration of 1 ml of 5% (v/v) acetic acid in 0.9% NaCl with an 8 cm long cannula in Day1 followed by 2% DSS orally in drinking water for 7 days

Group 3: was served to investigate the effect of intragastric administration of Spirulina fusiform in a model of ulcerative colitis. The tested algae was dissolved in distilled water forming suspension and administrated alone by stomach gavage in a
dose of 500 mg /kg for 7 days before induction of ulcerative colitis and resumed for the end of experiment in combination with DSS [9].

All through the experiments, stool of all animals was collected and evaluated every day for consistency and presence of blood by both naked eye and occult blood. The disease severity was evaluated using disease activity index (DAI) was taken before the start and end of experiment. At the last day of experiment, body weight of all animals was measured then they were sacrificed by cervical dislocation.

The abdominal cavity was opened and the entire colon was removed and rinsed with saline. Specimens of the colon were opened, examined, scored for visible mucosal damage. Tissue sample from each rat was obtained from area showing ulceration by naked eye. Each sample was divided into two parts one part was preserved in 10% formalin for histopathological examination the other part was weighed and homogenized then were centrifuged for 15 min at 17,000 rpm. The supernatants were collected and kept frozen at-80 °C for subsequent biochemical studies.

**Assessment of disease activity index (DAI)**

Body weight of each animal was measured immediately before the experiment starting (day 1) and just before scarification. DAI assessment is the combined of weight loss compared to initial weight, stools consistency, bleeding Scores were defined as follows:

- **weight loss:** 0 (no loss), 1 (1–5%), 2 (5–10%), 3 (10–20%), and 4 (>20%)
- **stools consistency:** 0–1 (normal), 2–3 (loose stools), and 4 (diarrhoea)
- **bleeding:** 0 (no blood), 1 (Hemoccult positive), 2–3 (Hemoccult positive and visual pellet bleeding), and 4 (gross bleeding, blood around anus) [10].

- **mucosal damage,** 0: no damage; 1: localized hyperaemia, but no ulcer or erosions; 2: ulcer or erosions with insignificant inflammation; 3: ulcer or erosions with inflammation at one site; 4: two or more major sites of ulceration and/or inflammation; 5: two or more major sites of ulceration and inflammation extending more than 1 cm along the length of the colon [11].
Biochemical assessment

Colon MDA assay

The extent of lipid peroxidation was determined as the concentration of thiobarbituric acid reactive substances (TBARS). The amount of MDA formed was measured spectrophotometrically at 532 nm as nmol per mg protein [12].

Colon SOD and CAT assays

Superoxide dismutase (SOD) and catalase (CAT) activities were measured spectrophotometrically at 435 nm (U/g tissue) according to manufactured instructions [13, 14].

Colon TNF-a assay

Colon TNF-a concentration was measured by ELIZA according to the principles described previously by [15].

Colon IL1B assay

Colon IL1B concentration was measured by ELIZA according to the principles described previously by [16].

Detection of occult blood in stools [17].

Histopathological assessment

The formalin-fixed specimens were embedded in paraffin, sectioned (5 mm), and stained with haematoxylin and eosin, and then sections were evaluated by light microscopy [18].

Statistical analysis

In the statistical comparison using IBM Corp. Released 2010. IBM SPSS Statistics for Windows, Version 19.0. Armonk, NY: IBM Corp. Program between the different groups, the significance of difference was tested using analysis of variance test (P value): used to compare the mean of more than two groups of quantitative data using multiple comparison post-hoc tests (least significant difference). P value less than 0.05 was considered statistically significant, whereas more than 0.05 was statistically insignificant. P value less than 0.01 was considered highly significant.

Results:

Intra-rectal single dose 1ml of 5%(v/v) acetic acid followed 2% dextran sulphate in drinking water produced marked loss of weight, loss of stool consistency, occult blood in stool and mucosal damage (table:1). They produced microscopic picture similar to chronic active colitis manifested by complete disruption of mucosal crypts, as well as sub mucous oedema and leukocyte infiltration, basal lymphocytosis.
and fibrosis (Figure : 2,3,4 ) Colonic tissue homogenates showed marked elevation of oxidative stress markers namely malondyde dehydrogenase , and reduction of antioxidant marker namely super oxide dismutase and catalase , On the other hand, inflammatory markers namely tumour necrosis factor –α and interleukin 1B were significantly elevated (table: 2 ).
Pre-treatment with spirulina normalized body weight and reduced indices of rectal bleeding, stool consistency and mucosal damage compared with non-treated group but such values are still above corresponding indices of normal control groups (table:1). This was associated with marked reduction of inflammatory signs on histopathological sections , partial improvement of tested oxidative stress and inflammatory marker compared with non-treated chronic colitis group which were still statistically significant compared with normal ones (table:2 ).

Table 1: Effects of spirulina(500 mg /kg for 7 days)on average values (m+- SE) total body weight (g), indices for rectal bleeding, stool consistency and colon mucosal damage in on acetic acid (1ml of5% single dose )followed by dextran sodium sulphate 2% in drinking water for 7 days induced chronic active colitis in male albino rats (n=8).

<table>
<thead>
<tr>
<th></th>
<th>body weight loss</th>
<th>Rectal bleeding</th>
<th>Stool consistency</th>
<th>Mucosal damage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>index</td>
<td>index</td>
<td>index</td>
<td>index</td>
</tr>
<tr>
<td>Normal control</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Non-treated dextran sodium sulphate induced ulcerative colitis</td>
<td>3.02+0.13*</td>
<td>1.6+0.27*</td>
<td>3.1+0.13*</td>
<td>3.01+0.16*</td>
</tr>
<tr>
<td>Spirulina treated dextran sodium sulphate induced ulcerative colitis</td>
<td>1.5+0.12* **</td>
<td>0.5+0.2* **</td>
<td>1.5+0.19***</td>
<td>0.5+0.2* **</td>
</tr>
</tbody>
</table>

*Significant compared with normal control group
* **Significant compared with non –treated dextran sodium sulphate induced chronic colitis group
Table 2: Effect of spirulina (500 mg /kg for 7 days ) on average of colonic tissue concentration (m±SE) malondhyde (nmol/g) ,catalase (U/g) and superoxide dismutase (U/g), tumour necrosis factor –α (Pg /ml) and interleukin 1-b (mg /mg) in acetic acid (1ml of5% single dose followed by dextran sodium sulphate 2%for 7days in drinking water induced chronic colitis in male albino rats (n=8).

<table>
<thead>
<tr>
<th></th>
<th>Non treated control</th>
<th>Non treated dextran sodium sulphate induced chronic colitis</th>
<th>Spirulina treated dextran sodium sulphate induced chronic colitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malondhyde</td>
<td>100+2.58</td>
<td>177.6+ 5.85*</td>
<td>124.9+3.56* **</td>
</tr>
<tr>
<td>Catalase</td>
<td>362.6+6.54</td>
<td>273.2+7.04*</td>
<td>359+5.94* **</td>
</tr>
<tr>
<td>Superoxide dismutase</td>
<td>180+ 4.59</td>
<td>115.7+2.21*</td>
<td>166.5+4.47* **</td>
</tr>
<tr>
<td>tumour necrosis factor –α</td>
<td>83.2+2.24</td>
<td>145.5+3.22*</td>
<td>95.4+2.05* **</td>
</tr>
<tr>
<td>Interleukin-1b</td>
<td>10+0.15</td>
<td>20.7+0.45*</td>
<td>15.5+0.23* **</td>
</tr>
</tbody>
</table>

* Significant compared with normal control group

** Significant compared with non –treated dextran sodium sulphate induced chronic colitis group

(Fig. 1). Colon sections of normal control rats showing normal colon epithelium with intact basement membrane (yellow arrow), normal crypts arrangement and normal submucosa (green arrow). H&E, X100.

(Fig. 2). Chronic active colitis showing crypt loss with interstitial neutrophilic infiltration (arrow) accompanied by hyperplasia of the crypt lining epithelium (arrowhead). H&E, X200.
Discussion:

Ulcerative colitis is an IBD characterized by inflammation of the colorectal mucosa. Deregulation in the immune response, with infiltration of leukocytes into the mucosal interstitium play an important role in its pathogenesis together with excessive production of ROS2. [19]

The aim of this study was to estimate the effectiveness of spirulina as a new, safe and
cheap approach in the treatment of ulcerative colitis.

In this work, a new animal model of chronic colitis similar to ulcerative colitis was performed. In this model, sequential course consisting of single intra-rectal acetic acid followed by dextran sodium sulfate in drinking water for one week. Previous models using intra-rectal acetic acid may produce acute colitis which is not similar to ulcerative colitis [19]. Its intra-peritoneal injection produces chronic colitis similar to ulcerative colitis but the mortality rate is very high. [20]. Moreover, the previously mentioned model was not coping with pathogenesis of ulcerative colitis as the causative agents produced free radical mediated damage of intestinal epithelium which is frankly different as regards its pathogenesis from immune mediated human ulcerative colitis. [21]. On the other hand, the use of dextran sodium sulfate as sole chemical inducer produced intestinal inflammation that morphologically and symptomatically resembles epithelial damage seen in human ulcerative colitis [22].

The advantages of such model is that it takes in consideration the immunological background of the disease as dextran is very antigenic polysaccharide. Its main disadvantages is high cost. Other immunologically based models namely intra-rectal installation of either carrageenan or trinitrobenzoate sulfonic acid, submucous injection of peptidoglycan polysaccharides are very expensive. The last method needs special skill [23].

In this study, rats with ulcerative colitis induced by acetic acid-DSS showed a significant decrease in body weights, results were in agreement with others [21] who report that interpreted these findings through direct DSS toxic effects on intestinal mucosa and indirect actions on the gastrointestinal tract arising from reduced food intake.

Results obtained have shown that rats with ulcerative colitis induced by DSS–acetic acid showed a significant increase in the stools consistency group, increase occult/gross rectal bleeding and mucosal damage score as compared with normal control.

These results are in agreement with others [9 & 24] who report increases in the clinical score activity and morphological mucosal damage score which confirm the severity and chronicity of colitis. Furthermore, a
marked increase in DAI was recorded in biopsies taken from UC patients.

In this present study, it was shown that the levels of colonic MDA in the colitis group were higher than the normal control group. These results were in agreement with others who observed that oxidative stress and its consequent lipid peroxidation are able to aggravate free radical chain reactions, disrupt the integrity of intestinal mucosal barrier and activate inflammatory mediators, resulting in increased colonic MDA contents, as shown in both human and experimental animal studies [25].

In this present study, it was showed that SOD activity significantly decreased in the colitis group. These results were in agreement with those observed others [25] who observed also that superoxide dismutase (SOD) decides the systemic protection against inflammation. SOD restrains the lipid peroxidation in the colon by eliminating free-radicals, converting superoxide into peroxide (H2O2). However, it was demonstrated that colonic mucosa Cu/Zn-SOD and, Mn-SOD levels were higher than the control levels in patients with inflammatory bowel disease [26].

In the present study, there was significantly elevated colonic TNF-a. These results were in agreement with Abdel-Daim et al[9]. who observed also acetic acid administration promoted colonic inflammatory pathways manifested through significantly elevated colonic TNF-a and over expression of colonic NF-kB.

In this study, showed that IL1B activity significantly increase in the colitis compared to control group. They may be probably through induction of gene expression of cyclooxygenase 2 enzyme responsible for synthesis of inflammatory prostaglandin. Moreover, demonstrated that interleukin 1 beta plays a crucial role in pathogenesis of wide variety of fibrotic diseases in lung, skin, and kidney through stimulation of differentiation of pericytes to myelofibroblast then to mature fibroblasts [29].

Some studies [30 & 31] showed that chronic dextran sodium sulphate (DSS)-induced colitis in mice significantly increased serum levels of IL-1β, IL-6, IL10, TNF-α, and IL-17.

In this study, it was proved that catalase activity significantly decreased in the colitis
group. These results were in agreement with previous studies [25 & 26].

The histopathological examination has observed that colon of diseased animal showed crypt loss with interstitial neutrophilic infiltration represent activity accompanied with hyperplasia of the crypt lining epithelium and attempts of epithelization of the mucosal surface epithelium associated with marked fibroblastic cells proliferation and mononuclear cell infiltration as sign of chronicity.

This study revealed that rats with ulcerative colitis induced by acetic acid -DSS treated with spirulina showed a significant increase in body weights, compared with an ulcerated group which is similar to previous studies [24].

In this study revealed that rats with colitis ulcerative induced by acetic acid -DSS treated with spirulina showed a significant improvement in stools consistency group, decrease occult/gross rectal bleeding and mucosal damage score as compared with an ulcerated group which is similar to previous studies [24 & 27].

In this study revealed that rats with colitis ulcerative induced by DSS –acetic acid treated with spirulina showed a significant reduced levels of MDA compared to the rats with acetic acid -induced colitis which is similar to previous studies [9 & 8] suggesting that spirulina successfully inhibited lipid peroxidation.

This study revealed that rats with colitis ulcerative induced by DSS –acetic acid treated with spirulina showed significantly increased SOD activity than colitis groups. These results were in agreement with others [9] who observed that spirulina has antioxidant activity.

This study revealed that rats treated with spirulina showed significantly increased catalase activity than colitis groups. These results were in agreement with what was observed before [9] that spirulina has antioxidant activity.

In this study revealed that rats treated with spirulina showed significantly decreased the activity of TNF-a in spirulina treated groups was significantly decreased than colitis groups. These results were in agreement with that study which observed that spirulina has anti-inflammatory activity through inhibition of transcription of nuclear factor KPa pathway and TNF [24].
Present study revealed that rats treated with spirulina showed significantly decreased the activity of IL1B than colitis groups. These results were in agreement with those that observed that spirulina has immunomodulatory activity [9].

The histopathological examination has showing partial regeneration colon epithelium with intact basement membrane normal crypts and mild oedema and inflammatory cell in the sub mucosa.

**Conclusion:**

In conclusion, tested drug partially ameliorate clinical and pathophysiological manifestation of ulcerative colitis in adopted animal model of the disease. Its proper role in therapeutic strategy of ulcerative colitis need evaluation its therapeutic effect both compared and combined with other ulcerative colitis therapeutic agents such as sulfasalazine and immunosuppressive agents as regards efficacy, safety, convenience and cost.

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