Experimental Study

The study of biochemical and histopathological effects of spirulina in rats with TNBS-induced colitis

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Abstract: Objectives: To evaluate the beneficial effects of spirulina on the treatment of experimental colitis. Background: Spirulina, a planktonic blue green algae from oascillateriaceae family, has anti-inflammatory, anti-oxidant, antitumor, anti-viral, and antimicrobial effects, rendering it a natural drug of prophylactic and therapeutic properties. The effects of spirulina on colitis are not known. Methods: Wistar rats weighing 200~300 g were used. Experimental colitis was created during anesthesia using the trinitrobenzene sulfonic (TNBS) acid. The rats were randomly divided into the 3 groups. In the group 1 (sham; n=8), saline was administered via oral gavage 7 days after 1 ml of rectal saline was administered. In the group 2 (experimental colitis+spirulina; n=8), 2 g/kg spirulina was administered via oral gavage 7 days after the rectal 1 ml TNBS was administered. In group 3 (experimental colitis; n=8), enema was administered via oral gavage 7 days after the rectal 1 ml TNBS was administered. Eight days after the instigation of TNBS colitis, the rats were sacrificed and blood and tissue samples were taken. Histopathologic and immunohistochemical evaluations were conducted, and malondialdehyde (MDA), advanced oxidation protein products (AOPP), catalase (CAT), total antioxidant status (TAS), and glutathione (GSH) levels were determined. Results: Inflammation on mucosa and submucosa, hemorrhage, necrosis, cellular infiltration and crypt abscess formation, immunoreactivity and tissue MDA levels were decreased in the experimental colitis+spirulina group when compared to the experimental colitis group (p<0.05). Conclusion: The results of the present study indicate the beneficial effects of spirulina on TNBS-induced inflammatory bowel disease (Tab. 6, Fig. 10, Ref. 40). Full Text in free PDF www.bmj.sk. Key words: microalgae, spirulina, experimental colitis, inflammatory bowel disease, nutrition.

Inflammatory bowel disease (IBD), including Crohn’s disease and ulcerative colitis, is a common idiopathic, chronic inflammatory condition, which affects the gastrointestinal tract (1). IBD is characterized by intestinal inflammation and mucosal tissue damage and also involves neuromuscular apparatus (2). The etiology of IBD remains unclear although genetic, environmental, immunologic, and dietary factors play a part (3). Recently, increased oxidative stress also has been supposed to play a role (4).

Spirulina, which grows naturally in warm climate of some Mexican and African countries is a planktonic blue green algae of the Oscillateriaceae family (5). Spirulina has been labeled as a powerful food, rich in proteins, carbohydrates, polyunsaturated fatty acids, sterols and some more vital elements like calcium, iron, zinc, magnesium, manganese and selenium. It is a natural source of vitamin B12, vitamin E, ascorbic acid, tocopherols and whole spectrum of natural mixed carotene and xanthophylls phytopigments (6).

At present, the drugs used for the treatment of inflammatory bowel disease are corticosteroids, immunomodulatory drugs, antibiotics, sulfasalazine, IgG anti-TNF-α antibody (7). Unfortunately, these drugs have serious side effects, which will limit their use (8). Spirulina species exert anti-inflammatory (9), antioxidant (10), anti-viral (11), antimicrobial (12) effects. The multifunctional role of Spirulina species makes it an ideal natural drug with immense prophylactic and therapeutic properties (13).

To the best of our knowledge, the activity of spirulina has never been investigated in the treatment of TNBS-induced experimental colitis. Therefore, this study was planned. Our aim was to determine whether or the activity of spirulina is useful in the treatment of TNBS-induced experimental colitis and to investigate its effect on enzyme activities in blood and bowel tissue in the inflammatory bowel disease.

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Materials and methods

Animals

Twenty-four Wistar rats, weighing 200–300 g each, were obtained from Gazi University Laboratory Animal Breeding and Experimental Research Center (Ankara, Turkey).

They were held in stainless-steel cages in the animal room, which was maintained on a 12 h light/12 h dark cycle at 21–22 °C. All rats were allowed free access to food and water. The structure of this study and animal experimental procedures were approved by the Ethical Committee of Gazi University.

TNBS-Induced Experimental Colitis

Experimental colitis was induced using TNBS as described by Morris et al (1989) (15) with slight modifications. In brief, rats were anesthetized i.m. with Ketamin hydrochloride (50–90 mg/kg). A baby-feeding tube was inserted rectally into the colon so that the tip was 8 cm proximal to the anus. Thereafter, 0.60 ml of TNBS 5 % w/v (40 mg: Sigma Chemical Co., St. Louis MO) in 0.25 ml of ethanol, resulting in a total volume of 0.85 ml, was instilled into the lumen of the colon, and the tube was flushed with 0.5 ml of air.

Experimental Design

TNBS/ethanol colitis was induced in 24 rats randomized into three groups.

Group 1 (Sham; n=8): saline was administered orally via gavage 7 days after the 1 ml rectal saline was administered

Group 2 (Experimental colitis+spirulina): 2 g/kg spirulina was administered orally via gavage 7 days after the rectal 1 ml TNBS was administered

Group 3 (Experimental colitis): enema was administered orally via gavage 7 days after the rectal 1 ml TNBS was administered

Eight days after the instigation of TNBS colitis, the rats were sacrificed and the colonic mucosal MDA, AOPP, CAT, TAS, GSH levels were measured. During sacrifice, the intracardiac route was used to collect blood samples and serum MDA, AOPP, CAT, TAS levels were measured.

Assessment of Antioxidant Status in Colonic Tissue

Tissue samples were homogenized in ice-cold 1/10 50 mM Tris-HCl (pH=7.4) buffer, and then centrifuged for 15 min at 15,000 rpm. After homogenization, MDA and AOPP were determined in supernatants.

Lipid Peroxidation Determination

The lipid peroxidation level in tissue samples is expressed by MDA. It was measured according to procedure of Ohkawa et al (1979) (16). The principle of this method is spectrophotometric measurement of the red color, which exists after binding MDA from proteins with dodecil sodium sulfate (SDS) and thiobarbituric acid (TBA) in the environment where pH is 3.5.

Serum MDA levels

Serum MDA level was measured according to procedure of Yoshioka et al (1979) (17). The principle of the method is based on the colorometric measurement of TBA and MDA complex formed after collapse of serum protein with trichloroaceticacid (TCA).

Serum/tissue AOPP levels Assay

Tissue and serum AOPP levels were measured according to spectrometric method by Witko-Sarsat (1996) (18).

Total Antioxidant status (TAS)in tissue/serum

Total Antioxidant status (TAS) was measured according to Cayman Antioxidant Assay Kit (Cat. No. 709001). Glutathione Activity was measured according to Cayman Glutathione Peroxidase Assay Kit (Cat. No. 703102). Catalase activity was measured according to Cayman Catalase Assay Kit (Cat. No. 707002).

Histopathological analysis

Light microscopy

Tissue samples were fixed in 10 % neutral buffered formalin after the conventional histological process, 4 μm thick slices were cut and stained with Hematoxylin and eosin. All slides were evaluated with Leica DM4000B light microscope (Leica, wetzlar, Germany).

The degree of inflammation was graded semiquantitatively. Histopathological analysis was performed with the scale previously used for experimental colitis by Gulec et al (2001) (19) in the Table 4.

Except for inflammation; Loss of mucosal architecture, cellular infiltration, crypt abscesses formation, goblet cell deple- tion analyses was performed By Giris et al (2008) (20) in the Table 5.

Immunohistochemical Method

Formalin fixed, paraffin embedded tissue sections, 5 micron thick, were incubated for one night at 37 °C and for 1 h at 60 °C. Xylol applications (15 min) were performed twice. The slides were then laid in 96 % absolute alcohol and 80 % ethanol for 10 min, followed by distilled water, twice for 5 min. They were boiled in high-temperature microwave oven in 10 % citrate buffer for caspase 3 immunoperoxidase. After 20 min at room temperature, the tissue was enrolled with a pap- pen (hydrophobic pen). After washing with distilled water and phosphate-buffered saline (PBS), hydrogen peroxide was added in drops. After washing with PBS, ultra V block (Cat.# TA-125-UB Thermo Fisher Scientific, Fremont, CA, 94539, USA) was applied. After a 1-h application of primary antibody including Caspase-3 (Rabbit Polyclonal Antibody Cat.#RB-1197-P, Labvision/NeoMarkers Corporation, Fremont, CA, USA), the samples were washed with PBS and a post-PBS level was applied (Biotinylated Goat Anti-Polyvalent and Streptavidin Peroxidase respectively). After washing with PBS, the specimens were placed in AEC (3-Amino, 9-Ethylcarbazole) (Cat.# TA-125-HA Thermo Fisher Scientific, Fremont, CA, 94539, USA) chromogen for 10 minutes. Finally, the counter stain with Mayer’s hematoxylin was performed for 2
Tab. 1. Effect of spirulina on TNBS induced changes in antioxidant parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sham</th>
<th>Experimental colitis (TNBS)</th>
<th>Experimental colitis+ spirulina</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (nmol/g tissue)</td>
<td>40.67±15.42</td>
<td>54.78±22.34</td>
<td>36.94±2.31*</td>
</tr>
<tr>
<td>AOPP (mmol/g tissue)</td>
<td>5.68±1.32</td>
<td>6.43±0.86</td>
<td>6.13±1.77</td>
</tr>
<tr>
<td>CAT (mmol/min/g tissue)</td>
<td>156.89±16.82</td>
<td>146.06±17.72</td>
<td>117.20±36.28**</td>
</tr>
<tr>
<td>TAS (mM trolox equivalent/g tissue)</td>
<td>85.50±18.52</td>
<td>107.38±32.11</td>
<td>123.75±28.51**</td>
</tr>
<tr>
<td>GSH (mmol/min/g tissue)</td>
<td>31.86±17.16</td>
<td>27.40±17.20</td>
<td>30.00±20.63</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD, * p<0.05 compared to experimental colitis, ** p<0.05 compared to sham.

Tab. 2. Effect of spirulina on TNBS induced changes in antioxidant parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sham</th>
<th>Experimental colitis (TNBS)</th>
<th>Experimental colitis+ spirulina</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (nmol/ml serum)</td>
<td>7.44±0.63</td>
<td>8.12±0.88</td>
<td>8.29±0.98</td>
</tr>
<tr>
<td>AOPP (µmol/L serum)</td>
<td>197.25±45.30</td>
<td>186.63±56.58</td>
<td>156.00±52.63</td>
</tr>
<tr>
<td>CAT (mmol/min/ml serum)</td>
<td>16.76±3.98</td>
<td>15.70±4.10</td>
<td>11.60±4.20*</td>
</tr>
<tr>
<td>TAS (mmol trolox equivalent)</td>
<td>2.41±0.82</td>
<td>2.09±0.76</td>
<td>2.61±0.33</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD, * p<0.05 compared to experimental colitis.

Tab. 3. Weight evolution (g) among the groups data are mean and standard deviation.

<table>
<thead>
<tr>
<th>Group</th>
<th>Weight before</th>
<th>Weight after</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental colitis</td>
<td>257.25±22.22</td>
<td>221.75±15.02*</td>
</tr>
<tr>
<td>Experimental colitis+ spirulina</td>
<td>320.00±28.58</td>
<td>291.88±25.00**</td>
</tr>
<tr>
<td>Sham</td>
<td>269.88±15.72</td>
<td>257.98±15.69***</td>
</tr>
<tr>
<td>Overall mean</td>
<td>282.38±35.22</td>
<td>257.17±16.67</td>
</tr>
</tbody>
</table>

* p<0.05 compared to weight before the experiment, ** p<0.05 compared to experimental colitis.

All hematoxylin slides were evaluated with Leica DM 4000 B light microscope (Leica, Wetzlar, Germany).

To prevent inter-individual bias, all tissues were evaluated by the same histologist (N.L.), who was blinded to the origin of the samples. The relative intensity of immunoreactivity staining was assessed quantitatively as previously described by McCarty et al (1985) (21), taking into account both the intensity and the distribution of a specific staining. A value of HSCORE (histological score) was derived from the sum of the percentages of positively stained cells multiplied by the weighted intensity of staining. HSCORE=Ωi(1-I), where i represents staining intensity (0=no expression, 1=mild, 2=moderate, and 3= intense) and Pi is the percentage of stained cells for each intensity.

Apoptosis evaluation with Caspase-3 was carried out by making measurements in five microscopic fields in each animal’s section in the experimental colitis, experimental colitis+ spirulina and sham group (Table 6).

Statistical Analyses

Results

Antioxidant activities were investigated through the levels of colonic and serum MDA, AOPP, CAT, TAS, GSH.

Table 1 demonstrates levels of colonic MDA, AOPP, CAT, TAS, GSH.

MDA levels were decreased in experimental colitis+ spirulina group when compared to the experimental colitis groups (p<0.05). No significant differences was seen in MDA level in experimental colitis+ spirulina group when compared to the sham group (p>0.05).

No significant difference was seen between experimental colitis+ spirulina group and sham group (p>0.05) in terms of AOPP level.

CAT activity was decreased in experimental colitis+ spirulina group when compared to the sham group (p<0.05). No signifi-
Tab. 4. The scale for light microscopic scoring.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal histological appearance</td>
<td>0</td>
</tr>
<tr>
<td>Inflammation on mucosa</td>
<td>1</td>
</tr>
<tr>
<td>Inflammation on mucosa and submucosa</td>
<td>2</td>
</tr>
<tr>
<td>Hemorrhage and necrosis</td>
<td>3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Grade</th>
<th>Sham (n=8)</th>
<th>Experimental colitis (n=8)</th>
<th>Experimental colitis +spirulina (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>8</td>
<td>—</td>
<td>3</td>
</tr>
<tr>
<td>1</td>
<td>—</td>
<td>—</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>—</td>
<td>5</td>
<td>—</td>
</tr>
<tr>
<td>3</td>
<td>—</td>
<td>3</td>
<td>—</td>
</tr>
</tbody>
</table>

A significant difference in colonic CAT activity was observed between experimental colitis+spirulina and experimental colitis group (p<0.05) However, the p value was 0.059.

TAS activity was increased in experimental colitis+spirulina group when compared to the sham group (p<0.05).

No significant difference in colonic TAS activity was observed in experimental colitis+spirulina group when compared to the experimental colitis group (p>0.05). However, TAS activity was increased in experimental colitis+spirulina group.

No significant difference in the colonic GSH level was observed in the experimental colitis+spirulina group when compared to the experimental colitis and sham group (p>0.05). However, GSH level was increased in the experimental colitis+spirulina group rather than in the experimental colitis group.

We observed that TNBS administration decreased the GSH level in the colon and Spirulina treatment prevented this depletion but it was not statistically different (p>0.05).

Table 2 demonstrates levels of serum MDA, AOPP, CAT, TAS.

Serum CAT activity was decreased in the serum experimental colitis+spirulina group when compared to the sham group (p<0.05).

No significant difference in serum CAT activity was observed in the experimental colitis+spirulina group when compared to the experimental colitis group (p>0.05) However, the p value was 0.059.

No significant difference was seen in serum MDA level in the experimental colitis+spirulina group when compared to the experimental colitis and sham group (p>0.05). MDA level was increased in experimental colitis+spirulina group in comparison to the experimental colitis group.

No significant difference was seen in serum AOPP level in the experimental colitis+spirulina group when compared to the experimental colitis and sham group (p>0.05). However, in experimental colitis+spirulina group when compared to the sham group, the serum AOPP p value was 0.52.

Serum AOPP level was seen to be decreased in experimental colitis+spirulina group compared to the experimental colitis group. 

Fig. 1. Sham group: forming temporary folds in surface epithelial cells (†), lumen (L), Lamina propria (LP), submucosa (SM), muscularis externa (ME) layers showing normal colonic mucosa appearance (HE, ×4 magnification).

Fig. 2. Sham group: normal epithelial cells (†), Lamina propria (LP), submucosa (SM) showing normal colonic mucosa appearance (HE, ×10 magnification).

Fig. 3. Experimental colitis group: Lumen (L), Lamina propria and submucosa showing intense lymphocyte infiltration appearance (*) (HE, ×4).
Colonic inflammation was assessed using a modification of the histopathologic grading system of Gulec et al (2001) (19) (Table 4).

There were significant differences (p<0.05) in the light microscopic scoring between the sham and the experimental colitis groups. Inflammation on mucosa, inflammation on submucosa, hemorrhage and necrosis were increased in the experimental colitis group when compared to the sham group (p<0.05).

There were significant differences (p<0.05) in the light microscopic scoring between the experimental colitis and experimental colitis+spirulina groups. Inflammation on mucosa and submucosa, hemorrhage and necrosis were decreased in the experimental colitis+spirulina group when compared to the experimental colitis group (p<0.05).

There were significant differences (p<0.05) in the light microscopic scoring between the experimental colitis+spirulina and the sham groups. Inflammation on mucosa was increased in the experimental colitis+spirulina group when compared to the sham group (p<0.05).

In comparison between sham and experimental colitis groups in terms of the light microscopy scoring system, in the experimental colitis group (Fig. 3), lymphocyte infiltration was found to be significantly increased compared to the sham group (Fig. 1) in the layers of lamina propria (LP), submucosa (SM) and muscularis externa (ME) (p<0.05).

Experimental colitis between the groups of experimental colitis+spirulina in terms of scoring the comparison of light microscopy

Experimental colitis+spirulina group (Fig. 5); lymphocyte infiltration, lamina propria (LP), submucosa (SM) and muscularis externa (ME) statistically significant decrease was observed in the experimental colitis group (Fig. 3) (p<0.05).

In the comparison of sham and experimental colitis+spirulina groups in terms of light microscopy scores, in the experimental...
Tab. 5. Effect of spirulina on histopathological scoring of TNBS induced colitis in rats.

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Loss of Mucosal architecture</th>
<th>Cellular infiltration</th>
<th>Crypt Abscess formation</th>
<th>Goblet Cell depletion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>0.14±0.40</td>
<td>0.30±0.50</td>
<td>0.00±0.00</td>
<td>0.14±0.40</td>
</tr>
<tr>
<td>Experimental colitis</td>
<td>1.00±0.00</td>
<td>3.00±0.40</td>
<td>1.00±0.00</td>
<td>0.88±0.40</td>
</tr>
<tr>
<td>Experimental colitis+spirulina</td>
<td>0.50±0.54</td>
<td>1.13±0.40* **</td>
<td>0.13±0.40*</td>
<td>0.75±0.50</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SD, * p<0.01 compared to the experimental colitis group, ** p< 0.01 compared to sham.

Tab. 6. Value of immunohistochemical score with Anticaspase-3.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>no expression</td>
<td>0</td>
</tr>
<tr>
<td>mild</td>
<td>1</td>
</tr>
<tr>
<td>moderate</td>
<td>2</td>
</tr>
<tr>
<td>intense</td>
<td>3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sham (n=8)</th>
<th>Experimental colitis+spirulina (n=8)</th>
<th>Experimental colitis (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.6</td>
<td>1.6</td>
<td>2.1</td>
</tr>
<tr>
<td>1.6</td>
<td>1.6</td>
<td>2.1</td>
</tr>
<tr>
<td>0.8</td>
<td>1.4</td>
<td>2.1</td>
</tr>
<tr>
<td>1.4</td>
<td>1.4</td>
<td>2.4</td>
</tr>
<tr>
<td>1.4</td>
<td>1.6</td>
<td>2.4</td>
</tr>
<tr>
<td>0.8</td>
<td>1.6</td>
<td>2.1</td>
</tr>
<tr>
<td>0.7</td>
<td>1.4</td>
<td>2.4</td>
</tr>
<tr>
<td>1.6</td>
<td>1.6</td>
<td>2.1</td>
</tr>
</tbody>
</table>

Colitis+spirulina (Fig. 6) group, lymphocyte infiltration was found to be significantly increased compared to the sham group in the layers of lamina propria (LP), submucosa (SM) and muscularis externa (ME) times the sham group (Fig. 2) (p<0.05).

Colonic inflammation was assessed using a modification of the histopathologic grading system of Daneshmand et al (2008) (22) (Tab. 5).

Cellular infiltration, crypt abscess formation was decreased in the experimental colitis+spirulina group when compared to the experimental colitis group (p<0.05).

Cellular infiltration was increased in the experimental colitis+spirulina group when compared to the sham group (p<0.05).

Loss of mucosal architecture, cellular infiltration, crypt abscess formation, Goblet cell depletion were seen to be increased in the experimental colitis group when compared to the sham group (p<0.05).

Apoptosis evaluation with Caspase-3 was carried out by making measurements in five microscopic fields in each animal’s section (Tab. 6).

No statistically significant difference was observed (p>0.05) in the immunohistochemical scoring between the sham and experimental colitis+spirulina groups.

There were significant differences (p<0.05) in the immunohistochemical scoring between the experimental colitis and experimental colitis+spirulina groups. Immunoreactivity was decreased in the experimental colitis+spirulina group compared to the experimental colitis group (p<0.05).

Fig. 7. Immunohistochemical appearance of sham group showing negative or mild immunoreactivity (Anti Caspase-3).

Fig. 8. Immunohistochemical appearance of Experimental colitis group showing moderate or strong immunoreactivity (Anti Caspase-3).
Immunohistochemical scoring assessment

In the evaluation of apoptosis with Caspase-3, while there was a negative or mild immunoreactivity in sham group (Fig. 7); in the experimental colitis+spirulina group there was a mild immunoreactivity (Fig. 10).

Moderate or strong degree of immunoreactivity was seen in the experimental colitis group, (Figs 8 and 9) A mild immunoreactivity was seen in the experimental colitis+spirulina group (Fig. 10).

While in the experimental colitis group, moderate or severe immunoreactivity was seen (Fig. 8 and 9), in the experimental colitis+spirulina group a mild immunoreactivity was seen (Fig. 7).

Discussion

Animals with TNBS model colitis respond to drugs that have been proven useful in IBD (23). The histological features of the animals receiving TNBS were chronic inflammation, relatively long duration of inflammation, and changes in various inflammatory mediators. Thus, the model is rather suitable for the assessment of the effects of potential agents (24). Therefore, we used TNBS-induced colitis model.

The colitis was induced by TNBS as histologically the inflammatory response includes mucosal and submucosal infiltration by lymphocytes, macrophages, polymorphonuclear leukocytes, connective tissue mast cells, and fibroblasts. Segmental ulcerations, mucosal edema, bleeding and necrosis are common in this model (15, 25). In our study, we successfully produced experimental colitis with TNBS.

The results of our study has shown that light microscopic evaluation revealed a decreased lymphocyte infiltration, immunohistochemical evaluation revealed a decreased immunoreactivity, histopathological evaluation a decreased cellular infiltration and crypt abscess in the treatment group (experimental colitis+spirulina). This shows the activity of spirulina in experimental colitis induced by TNBS.

This is the first study to demonstrate the healing effects of spirulina on experimental colitis.

In literature, we can find few trials of different algae species.

Matsumoto et al (26) have shown that Cladosiphon okamuranus tokida ameliorates murine chronic colitis through the down-regulation of interleukin-6 production on colonic epithelial cells and may be useful as a dietary substance for the patients with IBD.

In another study, Dvir et al (27) studied the effect of red microalgal Porphyridium sp. on gastrointestinal physiology and lipid metabolism, suggested that this algae can regulate gastrointestinal physiology and lipid metabolism and may be useful as a functional food.

In another study, Gonzalez et al (28) studied the anti-inflammatory activity of phycocyanin extract in acetic acid-induced colitis and reported that intestinal myeloperoxidase enzyme levels, which increase in control colitis group, remarkably decreased in response to algae extract and inhibit inflammatory cell infiltration with histopathological and ultrastructural studies.

In another study, Sakai et al (29) described that Sargassum horneri, a marine brown algae, increases CL absorption in isolated rat colon by activation of leukotrienes.

A depletion of GSH levels in experimental colitis rat model was previously reported by Sánchez de Medina et al (30) and Girix et al (31).

In our study, tissue GSH level was also decreased in experimental colitis group but there was no significant difference (p>0.05).

Blau et al (32) in their study, have shown that treatment with A cationized catalase was unable to prevent GSH reduction. In our study, tissue GSH level was also increased in the treatment group (experimental colitis+spirulina) but this increase was not significant (p>0.05).

Cetinkaya et al (33) in their study have shown that L-carnitine could be beneficial in the treatment of colitis. CAT activity was seen to be significantly decreased in the treatment group (experimental colitis+spirulina group) when compared to the sham (p<0.05) group. Similarly, in the present study, we found
that CAT activity was significantly decreased in the treatment group (experimental colitis+spirulina group) when compared to the sham (p<0.05).

Dost et al (34) in their study shown that Hypericum perforatum had a protective effect on TNBS induced colitis. At the end of the study no significant difference was found in CAT activity between experimental colitis+spirulina group and experimental colitis group (p>0.05).

In our study, no significant difference in colonic CAT activity was found among the groups.

In literature review, we did not find any increase or decrease of AOPP with an administered agent in rats with experimental colitis. In the present study, no significant difference was found between the groups in terms of AOPP.

It has been proposed that lipid peroxidation mediated by oxygen free radicals plays an important role in the pathogenesis of IBD Pelli et al (35). MDA accumulation in tissues is an indicative of the extent of lipid peroxidation and oxidative stress, Halliwell et al (36). It has been shown that MDA levels were decreased with treatment Gulce et al (37), Dong et al (38), Giris et al (20).

Bedirli et al (39) in their study shown that chlorella was effective in inhibiting intestinal mucosa lipid peroxidation.

In the present study, intestinal MDA levels were found to be significantly changed between the treatment (experimental colitis+spirulina) and experimental colitis group, indicating that spirulina is effective against lipid peroxidation.

Dost et al (34) in their study have found that HP had a protective effect on TNBS induced experimental colitis. They did not find any significant difference in plasma MDA level between groups (p>0.05).

In the present study, no significant difference was found between the groups (p>0.05) in terms of plasma MDA level.

We have observed significant decrease in body weight in experimental colitis group (p<0.05), while no significant decrease in body weight was observed in the experimental colitis+spirulina group (p>0.05).

Salman et al (40) in their study observed that Chlorella extract increases weight gain in malnourished rats.

In conclusion, the present study indicates the efficacy of spirulina in TNBS-induced inflammatory bowel disease.

We think that this effect of spirulina is due to its anti-oxidant, Prenkumar et al (13), anti-inflammatory, Reddy et al (9) properties and a high level of nutrients.

References


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