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Original Research Article

SYNERGISTIC AMELIORATIVE EFFECT OF *Lactobacillus* AND *Spirulina platensis* AGAINST EXPERMINTAL COLITIS IN ALBINORATS: ANTIOXIDANT, HISTOPATHOLOGICAL AND MOLECULAR STUDIES

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Abstract: Ulcerative colitis (UC) considers one of inflammatory disorders which affect colon mucosa cause a substantial burden on human day life. In the past, treatment of UC depended on aminosalicylates and antibiotics but due to their adverse side effects and incomplete effectiveness, antioxidant anti-inflammatory agents are used nowadays to ameliorate UC. The aim of this work is to evaluate the modulatory effect of *Lactobacillus* and/or *Spirulina* oral administration in acetic acid induced colitisin albino rats. Rats were divided randomly into (6) groups. 1stgroup (negative control), 2nd group (acetic acid), 3rdgroup (Mesalazine) at dose 20mg/kg orally was used as positive drug control. 4th group (*Lactobacillus*) at dose1×10^9 CFU/rat daily. 5th group (*Spirulina*) at dose500mg/kg daily. 6thgroup (*Lactobacillus* at dose 1×10^9 CFU/rat + *Spirulina* at dose 500mg/kg). Results revealed that the experimental colitis group showed significant increase in DAI, macroscopic damage, colon weight, colonic MDA, NO, molecular expressions (iNOS and COX-2) and significant decrease in colon length, GSH level and CAT activity. *Lactobacillus* and/or *Spirulina* supplementation revealed significant improvement in macroscopic and microscopic finding, increase antioxidant biomarkers, significant inhibitions of MDA and nitric oxide. Furthermore, significant decline in COX-2 and iNOS expressions were reported. In conclusion, the protective effects of *Lactobacillus* and/or *Spirulina* in UC are due to their ability to reduce iNOS and COX-2 expressions, increase antioxidant biomarkers and significant inhibition of lipid peroxidations. Furthermore, *Lactobacillus* and *Spirulina* have synergistic protective effect on colon tissue and could be used in combination to ameliorate UC.

Key words: colitis; acetic acid; *Lactobacillus*; *Spirulina platensis*; Mesalazine; antioxidant, anti-inflammatory.

Introduction

Inflammatory bowel disease (IBD) is a an inflammatory disorder of the gastro intestinal tract, including ulcerative colitis (UC) and Crohn's disease(CD) (1). Ulcerative colitis is restricted to the colon mucosa, while any part of the whole gastrointestinal tract can be af-

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fected in Crohn's disease (2). It's occurrence and prevalence are common in any age and different area around the world, that means its emergence global disease(3). Ulcerative colitis pathogenesis is not completely understood. There are many factors affected these diseases, including immune genetic factors (4), abnormal micro biota (5), epithelial barrier disruption, broken of intestinal microbiota and other environmental factor (6).

The most clinical signs of colitis are abdominal tenderness, bloody mucous stool, purulent stool, and relapse. Furthermore, diarrhea, interrupted digestion, loss of body weight and an extensive burden on daily life (7). These clinical manifestation were established by scoring of disease activity index (DAI) (5).

Acetic acid is considered one of the main chemical widely used animal model to induced ulcerative colitis (8). Pathogenesis, histopathological features and inflammatory mediator profile to this type of colitis is phenotypically more identical to human IBD (9).

Specialized intestinal epithelial cells (IECs) are considered the physical barrier of luminal microbiota and has important role in maintaining intestinal homeostasis. So, any disturbance in the epithelial layer and intestinal permeability lead to dysregulated intestinal immune homeostasis and lead to IBD (2). Ulcerative colitis induced an increase reactive free radicals inductions and pro-inflammatory cytokines production. Furthermore, it showed significant intestinal epithelial cells apoptosis which disintegrate intestinal mucosal and barrier function. At the same time, inducible nitric oxide synthase (iNOS) (10) and cyclooxygenase-2 (COX-2) production are increased that playing a critical function in the incidence of this disease (11).

Probiotics known as "live microorganisms when supplemented in sufficient numbers, they induce health benefits to the host "(12). *Lactobacillus* is considered one of probiotics species which improving the component of intestinal micro flora(13), preventing action on IBD, and relieving colic symptoms(14). Furthermore, *Lactobacillus* has pronounced antimicrobial effect (15) and may change gut pH by producing some acids which prevent growth of another pathogenic bacteria(16).

Spirulina platensis is one of marine blue green microalgae with high nutritional values(17). It is rich source for many minerals, vitamins, protein and antioxidant anti-inflammatory compounds such as carotenoids, and phycocyanin pigment (18).Regarding to several effects of *spirulina*, it includes anticancer (19), antiviral (20), anti-allergic (21), antimutagenic (22), cytoprotective (23) and cardioprotective effects (24). Moreover it induces blood vessel-relaxing effect (25), hypocholesterolemic effect(26), hypolipidemic actions (27). In addition, hepatoprotective (28), neuroprotective, reduced concentrations of tumor necrosis factor (TNF-alpha) (29)and immune-enhancing action (30) were also reported.

In the past, treatment of UC depended on aminosalicylates, antibiotics, steroids, and immune modulators but incomplete effectiveness and their adverse side effects, natural antioxidant anti-inflammatory agents are used nowadays to ameliorate UC (10).

For that reason, the present work aimed to judge the modulatory effects of oral administration of *Lactobacillus* and/or *Spirulina platensis* in experimental colitis models in rats by estimating antioxidant parameters as well as molecular and histopathological investigations.

Materials and methods

Chemicals

Lactéal fort (*Lactobacillus* LB) capsules purchased from Tenth of Ramadan for pharmaceutical industries &diagnostic reagents (Rameda), Egypt. *Spirulina* purchased from the Algal unit of Biotechnology (National Research Center, Dokki, and Cairo, Egypt). Marsalaz tablet purchased from Marci pharmaceutical industries El- Obour City, Egypt. Acetic acid obtained from El-Nasr Pharmaceutical Chemicals Company (Cairo, Egypt). Diethyle ether obtained from (spinreact) Spin. EDTA from (Salix). Malondialdehyde (MDA), Catalase (CAT), Nitric oxide (NO) and Glutathione reduced (GSH) purchased from BIO-

DIGNOSTIC Company kits-Egypt. All chemicals utilized in this study was of analytical grade.

Animals and feed management

Forty-nine male albino rats of average weight (100 g/rat) were purchased from Animal House Colony of the Tanta Center. The rats were adapted to standard laboratory conditions (temperature 22–25°C, relative humidity 50–60%), rats were fed a balanced diet and water *ad libitum*.

Experimental design

Rats after adaptation period (2 weeks) were distributed randomly into six (6) groups (8 rats in each except control positive 9 rats). The 1st group (negative control), received saline orally for 10 days then followed by normal saline instillation at tenth day per rectum. The 2nd group (positive control), received saline orally for 10 days followed by 2ml of acetic acid (4%) intrarectally (10)at tenth day. The 3rd group (Mesalazine group) received 2ml acetic acid (4%) rectal instillation on day 10, then given Mesalazine orally at dose (20mg/kg) (31)for 5days after induction of colitis. The 4th group (*Lactobacillus* group) received daily *Lactobacillus* at dose (1×10^9CFU) orally/rat (14)for 10 days, then rectal instillation of 2ml acetic acid (4%) at tenth day. The 5th group (*Spirulina* group) received *Spirulina* daily at dose (500mg/kg) orally(32)for 10 days, then rectal instillation of 2ml acetic acid (4%) at tenth day. The 6th group (*Spirulina* + *Lactobacillus*) (combination group) oral received both *Spirulina* (500mg/kg) and *Lactobacillus* (1×10^9CFU) daily for 10 days, then rectal instillation of 2ml acetic acid (4%) at tenth day. All groups were observed daily for clinical signs, mortality rate, body weights, food and water intakes of the rats from each group. The rats were sacrificed at 5thday from induction of colitis, rats were slaughter under diethyl ether anesthesia. Colon segments were dissected, removed adipose tissue, washed with normal saline, for macroscopic and microscopic examination. Colon samples were preserved immediately at-80°C for molecular and oxidative stress analysis.

Induction of colitis

Colitis was produced by intra-colonic administration of 4% acetic acid (2ml)(33). Through a lubricant rectal pediatric urinary catheter under low-dose of ether anesthesia. Briefly, animals were restricted from food for 24h (34). The instillation site was about 8cm from the anal margin into the rectum. After instillation directly rats were maintained in vertical position for about 1 min to prevent acid leakage (35). Animals in the group one exposed to the same practice with saline instead of the acetic acid solution.

Assessment of disease activity index

According to (36)**,** clinical signs in colitis were determined by evaluating the disease activity index (DAI)(table 1). The disease activity index includes (stool consistency, presence of rectal hemorrhage and decrease in body weight), DAI in all the tested groups were observed daily after induction of colitis by 4% acetic acid.

Assessment of scoring severity of colitis

For macroscopic damage score (Table 2), the colon was examined visually either for adhesions or gross morphological alterations immediately after death (37).

Determination of colon weight and colon length

After separated the colon from adipose tissue and remind intestine must be determine the colon weight (g) and colon length (cm).

Tissue sample for histopathology and antioxidant biomarkers

Tissue specimens of colon were rapidly taking and equally divided into three parts : proximal part for molecular investigation (rapidly stored at -80°C) (38)**,** middle part used for histopathology, and distal part of colon stored at -20°C and used for oxidative stress and antioxidant parameters.

Estimation of antioxidant biomarkers of colon tissue homogenates

Prior to dissection, distal part of colon were rinsed with phosphate buffered saline (PBS) solution, pH 7.4, containing 0.16 mg/ml heparin to get rid of any red blood cells and clot. Then tissue homogenize in 5-10 ml buffer (i.e. 50 Mm potassium phosphate pH 7.4, 1Mm EDTA and 1 ml/l triton x-100 /g. tissue and Centrifuge at 4.000 rpm for 15 min. at 4^{o}C. The supernatant was separated into an Eppendorf tube and was preserved at −80^{o}C into aliquots for the spectrophotometric analysis of lipid peroxidation content by Malondialdehyde (MDA)(39), nitric oxide (NO) (40), Catalase (CAT)(41), and reduced Glutathione (GSH) estimation (42).

Detection of gene expression quantitatively by real time PCR

Forward and reverse primers sequence for *iNOS*, *COX2* and *β-actin* genes are presented in Table 3**.** Tissue RNA was extracted with Trizol (total RNA isolation reagent, iNtRON Biotechnology, Inc). Complementary DNA (cDNA) was synthesized by using Oligo (dT) primer HiSenSripte TMRH cDNA synthesis kits (IntROn) as described by the manufacturer's directions. The SYBR green was performed using BIoRad IQ2 (Japan) and the following protocol was used (43).The mRNA expression levels were normalized using β-actin.

Histopathological studies

Histopathological tissues preparation and examination was done according to (44)using H&E.

Statistical analysis

Data were presented as means ± S.E. using one-way ANOVA followed by Newman-keuls multiple comparisons using graph pad prism 7 software. Statistical significance was acceptable to a level of $P \leq 0.05$

Results

Mortality rate

Control negative group, fed on standard diet, showed no abnormal clinical signs or mortality during the whole period of experimental. Mortality rate showed in table 4. Generally, the control positive group revealed obvious increase in mortality rate (4/9) (44.4%). Meanwhile, administration of *Lactobacillus* alone decreased mortality rate (2/8) (25%). *Spirulina* alone also decrease mortality rate (2/8) (25%). Furthermore, the co-administration of *Lactobacillus* with *Spirulina* led to pronounced decrease in mortality rate (1/8) (12.5%), similar to Mesalazine group which used for ulcerative colitis treatment.

Disease activity index

The effect of *Lactobacillus* and/or *Spirulina* in experimental colitis on DAI was explained in figure1. Data demonstrated significant ($p \leq 0.05$) elevation in DAI in acetic acid group as compared with control one. Mesalazine group revealed insignificant decrease in DAI in comparison with control positive group. Similarly, *Lactobacillus* group caused pronounced decrease in DAI but this reduction was still insignificant as contrasted with control positive group. Meanwhile, *Spirulina* alone or in combination with *Lactobacillus* revealed significant ($p \leq 0.05$) improvement in DAI as matched with control positive group.

Scoring severity of colitis

The effect of *Lactobacillus* and/or *Spirulina* on macroscopic damage score was illustrated in figure 2. Data explored that significant ($p \leq 0.05$) increase in damage score in acetic acid positive colitis as compared with the negative none treated one. Whereas, all other treated groups reported significant ($p \leq 0.05$) reduction in score damage in comparison with the positive control group.

Colon weight and length

The effect of *Lactobacillus* and/or *Spirulina* on colon weight and colon length was showed in table 4 and figure 1. Concerning to colon

weight, the obtained data illustrated significant ($p \leq 0.05$) increase in the control positive group as contrasted with control negative one. On the other hand, Mesalazine group showed a decrease in colon weight but still insignificant as matched with control positive group. At the same time, simultaneous supplementation of *Lactobacillus* and/or *Spirulina* in acetic acid (4%) induced colitis revealed a significant ($p \leq 0.05$) improvement in colon weight as compared with control positive group. Furthermore, control positive group showed significant ($p \leq 0.05$) decrease in colon length in comparison with control negative group. Meanwhile, Mesalazine group revealed a significant ($p \leq 0.05$) increase in colon length as contrasted with control positive group. Similarly, a significant ($p \leq 0.05$) increase in colon length was detected in *Lactobacillus* and/or *Spirulina* groups as matched with control positive group.

Lipid peroxidation and antioxidant biomarkers:

The effect of *Lactobacillus* and/or *Spirulina* on Lipid peroxidation and antioxidant biomarkers were portrayed in figure 2. The data illustrated a significant ($p \leq 0.05$) increase in MDA and nitric oxide in colon tissue homogenate in the control positive group as compared with control negative one. Meanwhile, control positive group showed marked decrease in GSH and CAT but this decrease still statically insignificant in comparison with control negative group. Nevertheless, Mesalazine group revealed a significant ($p \leq 0.05$) decrease in MDA and NO content as contrasted with control positive group. Meanwhile, treatment by Mesalazine revealed increase in GSH and CAT but still insignificant as matched with control positive group. Regarding, *Lactobacillus* or *Spirulina* supplementation in acetic acid (4%) induced colitis group showed a significant ($p \leq 0.05$) decrease in colon tissue content of MDA and NO as compared with control positive group. Meanwhile, increase in GSH and CAT were observed but still insignificant in contrast with control positive one.

Similarly, the co-administration of *Lactobacillus* with *Spirulina* revealed a significant ($p \leq 0.05$) decline in MDA and NO as contrasted with control positive group. On the other hand, obvious enhancement in GSH and CAT were observed as matched with control positive group.

Molecular investigation

The effect of *Lactobacillus* and/or *Spirulina* in acetic acid (4%) induced colitis on the quantitative gene expression by real time PCR were illustrated in figure 2. The data reflect a significant ($p \leq 0.05$) increase iniNOS and COX2expressionin the control positive group as matched with the control negative one. On the contrary, Mesalazine group showed a significant ($p \leq 0.05$) reduction in iNOS and COX2 expression as compared with control positive group. Gastric intubations of *Lactobacillus* or *Spirulina* showed a significant ($p \leq 0.05$) improvement in iNOS and COX2expressionin comparison with control positive group. At the same time, simultaneous administration of *Lactobacillus* with *Spirulina* showed a significant ($p \leq 0.05$) decline in iNOS and COX2 expression as contrasted with control positive group.

Histopathological findings

The effect of *Lactobacillus* and/or *Spirulina* in acetic acid (4%) induced colitis on histopathological features was showed in figure 3. Colon of rats in control negative group demonstrated normal epithelium, intestinal glands and intestinal lumen. Colon of rats in none treated positive group (severe colitis) revealed necrosis of surface epithelial and enterocytes, deposition of irregular collagen fibers, crypt distortion, and loss of intestinal glands, mononuclear cell infiltration and apoptosis of mononuclear cell. Colon of rats in the Mesalazine group (colitis) revealed patchy mucosal necrosis, proliferation of submucosal lymphoid elements such as, lymphocytes, macrophages and plasma cells with submucosal edema and necrosis together mononuclear cell infiltration. Colon of rats in the *Lactobacillus* group (moderate colitis) showed mono-

nuclear cell infiltration in the mucosa and submucosa, in addition to, congestion of submucosal blood vessels and submucosal edema. Meanwhile, *Lactobacillus* group showed cell infiltration between the mucosal glands with necrosis of the surface enterocytes. Colon of rats in *Spirulina* group (mild colitis) showed mononuclear cell infiltration in the mucosa and between the mucosal glands, edema in tunica muscularis, and normal surface epithelium. Colon of rats in *Lactobacillus* with *Spirulina* group (mild colitis) showed only mononuclear cell infiltration between the mucosal glands with normal surface epithelium.

Table 1: Scoring of disease activity index (DAI)

Score	Weight loss %	Stool consistency	Occult/gross bleeding
0	0	Normal	Normal
1	1-5%	-	-
2	5-10%	Loose stools	Occult blood
3	10-15%	-	-
4	> 20%	diarrhea	Gross bleeding+ mucous

Table 2: Primers used for qPCR

Gene	primer sequence (5/ ------3/)	Reference
iNOS	F:CCTCCTCCACCCTACCAAGT R: CACCCAAAGTGCTTCAGTCA	(Villarán et al., 2010)
COX2	F:TGCGATGCTCTTCCGAGCTGTGCT R:TCAGGAAGTTCCTTATTTCCTTTC	(Bhatia et al., 2008)
β-actin	F: TGTGATGGTGGGAATGGGTCAG R: TTTGATGTCACGCACGATTTCC	(Villarán et al., 2010)

Table 4: colon lesion parameters (mean ± SE) in control and different treated groups

Groups / parameters	C-ve	C+ve	M	L	S	L+S
Mortality rate%	0%	44.4%	12.5%	25%	25%	12.5%
Body weight change	104.2±2.392^{a}	81.6±1.97^{b}	103±2.43^{a}	102.9±1.96^{a}	93±2.77^{a}	95.5±3.6^{a}
Colon weight	1.28±0.09^{b}	1.7±0.15^{a}	1.45±0.08^{ab}	1.36±0.08^{ab}	1.43±0.13^{ab}	1.4±0.04^{ab}
Colon length	14.54±0.20^{a}	12±0.58^{c}	13.2±0.2^{b}	13.75±0.48^{ab}	13.67±0.33^{ab}	13.8±0.37^{ab}
weight/length ratio	0.09±0.01^{b}	0.15±0.02^{a}	0.11±0.0^{b}	0.10±0.01^{b}	0.11±0.02^{b}	0.10±0.01^{b}

C-ve: Control negative, C+ve: Control Positive, (M): Mesalazine group, L: *Lactobacillus* group, SP: *Spirulina* group, L+SP: *Lactobacillus* +*Spirulin*a. Data were statistically analyzed as mean ± SEM. Rows carrying different superscript letters are significantly different at $p \leq 0.05$.

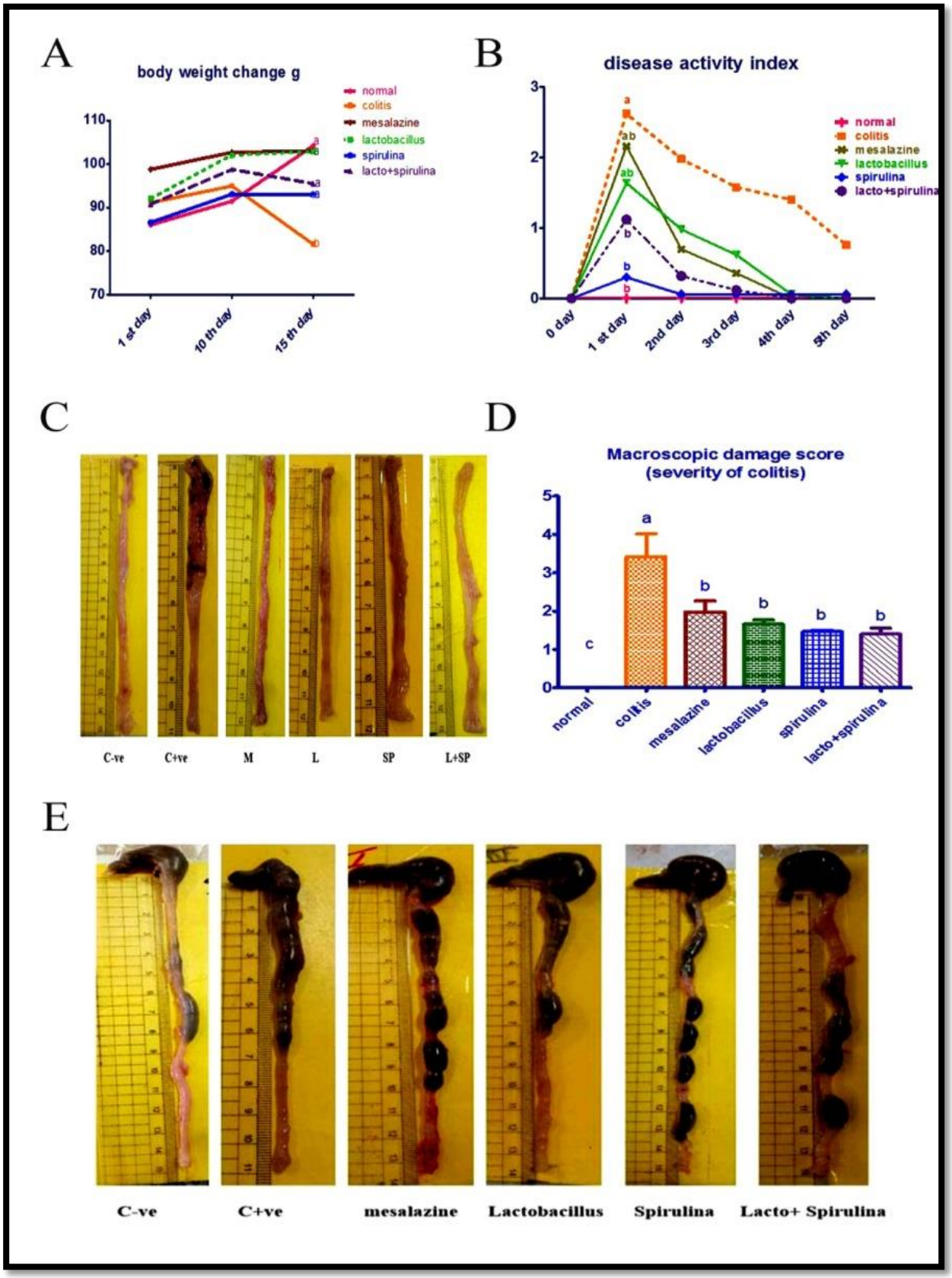

Figure 1: A) refer body weight change (g), B) disease activity index, C) Macroscopic damage in control and different treated groups, D) Colon length in control and different treated groups

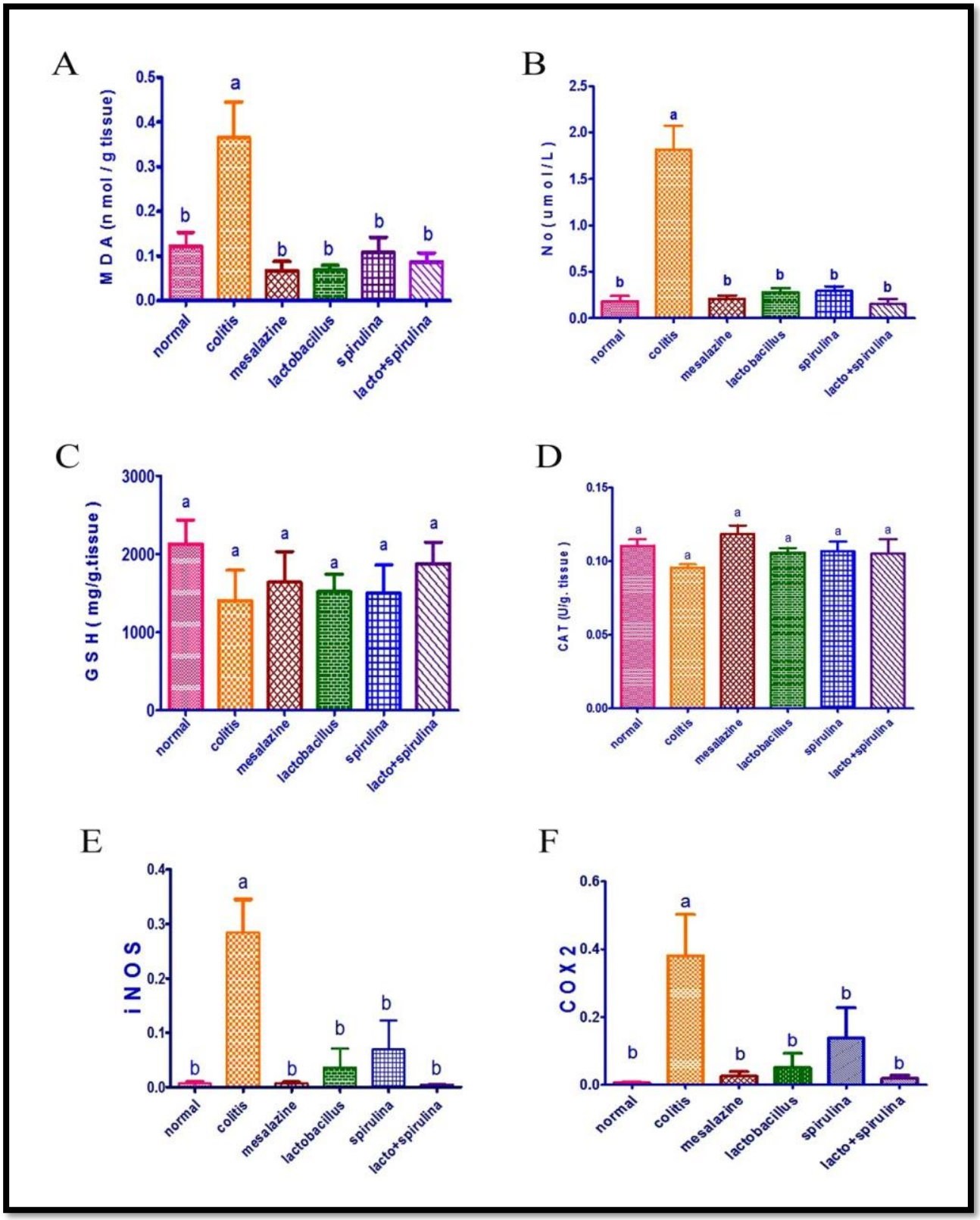

Figure 2: A, B) Lipid peroxidation, C,D) antioxidant biomarkers and E,F) molecular gene expression in control and different treated groups. Data were statistically analyzed as mean ± SEM. Rows carrying different superscript letters are significantly different at $p \leq 0.05$

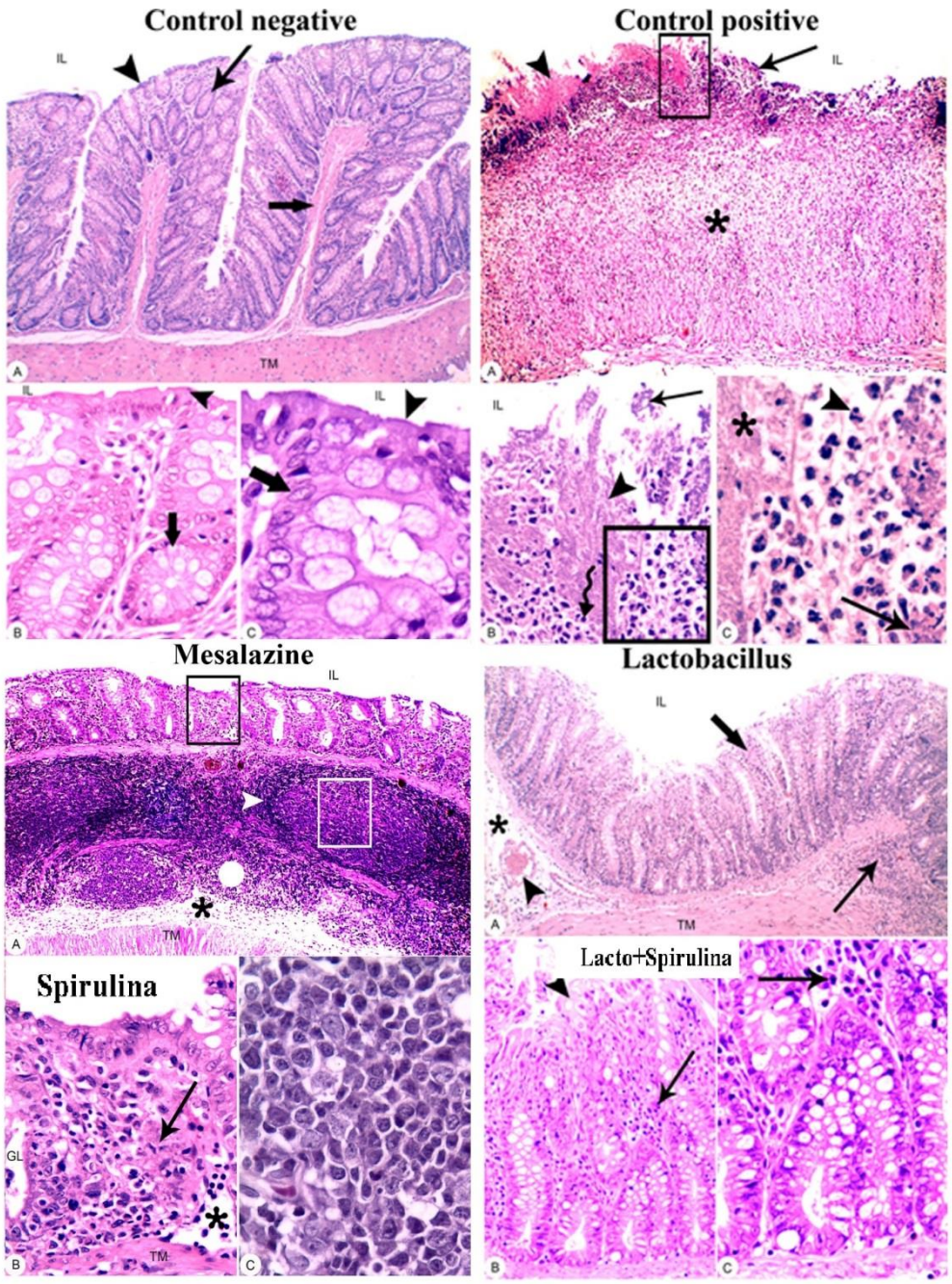

Figure 3: Histopathological feature in control and different treated groups. Microscopic features of colon rats by H&E stain. 1. Normal group, normal histologic architectures. 2. Acetic acid group (acute chemical colitis): showing necrosis of epithelial surface with deposition of irregular collagen fibers, crypt distortion, and loss of intestinal glands. 3. Mesalazine treated group, colitis: showing patchy mucosalnecrosis, proliferation of submucosal lymphoid elements (lymphocytes, macrophages and plasma cells), and submucosal edema.4. *Lactobacillus* treated group moderate colitis: showing mononuclear cell infiltration in the mucosa and submucosa, congestion of submucosal blood vessels and edema. 5. *Spirulina* treated group, mild lymphocytic colitis: showing mononuclear cell infiltration in the mucosa, edema with normal surface epithelium. 6. *Lactobacillus+Spirulina* treated group, mild lymphocytic colitis: showing mononuclear cell infiltration in the mucosa, with normal surface epithelium

Discussion

Ulcerative colitis is an inflammatory condition which cause abdominal pain, bloody diarrhea and mucous in stool(7). Furthermore, weight loss and other many symptoms that differ from person to person and it may be lead to colorectal cancer (45). The study designed to evaluate the effect of some natural agents (*Lactobacillus* and/or *Spirulina*) to ameliorate the clinical manifestation of acetic acid (4%) induced colitis in rats.

The present study exhibited significant increase in DAI and macroscopic damage in control positive rats as compared with the normal negative one. This increase could be referred to loss of appetite, decrease food consumption and feverish conditions which resulted in body weight reduction(46). Others referred this increase in DAI due to high inflammatory response and occasional ulceration that induced bloody diarrhea as confirmed by elevated mortality rate percent (44.4%), histopathological lesions and high macroscopic damage score and these results parallel with (9, 47).

At the same time, rats of control positive group showed significant increase of colon weight with significant reduction in colon length as compared with non treated one that might be referred to cellular swelling which resulted from shift of extracellular water into the cells associated with neutrophils and macrophage infiltration. Furthermore, cells turgor, submucosal edema, vascular dilatation and goblet cell hyperplasia. Similar result was obtained by(48, 49).

Oxidative stress and lipid peroxidation play acritical role in pathogenesis of ulcerative colitis (50, 51). Control positive animals revealed marked reduction in CAT and GSH, meanwhile, a significant elevation of MDA and NO was recorded as contrasted with control negative group. These results run parallel with those obtained by (52)

Generation of ROS and free radicals from migrated neutrophils attack the cellular macromolecules and lead to epithelial cell disruption with extensive colon damage(53, 54). Furthermore, ROS lead to massive oxidation of cell membrane phospholipids, proteins, and DNA. This oxidation cause further stimulates of more neutrophils and macrophage infiltration to damaged tissue (49).Consequently, intestinal mucosa to regulate ROS levels, have enzymatic and non-enzymatic complex antioxidant defenses mechanism such as reduced glutathione (GSH) and catalase (CAT), which try to heal and repair the damaged cells. Moreover, GSH and CAT were consumed by inflamed colon tissues to neutralize oxidative stress(55, 56).

Malondialdehyde (MDA) considered the end result of lipid peroxidation which caused by ROS. Based on that, the elevated MDA in acetic acid (4%) induced colitis rats referred to the increased lipid peroxidation and high cell damage(57, 58).

Nitric oxide (NO) is produced by some inflammatory cells such as granular leukocytes (neutrophils) or granular leukocytes (monocytes, and macrophages) as well as extravascular compartment as epithelial cells from inflamed colon tissue. Thereby, elevated NO content in experimental colitis considered as an index of inflammation(59).

Molecular genes expressions (iNOS and COX-2) are considered important gene expressions in acetic acid induced colitis, and they have synergistic effect to augmented the inflammatory reaction(60). Furthermore, recent investigations reported close interrelationship existing between iNOS and COX-2 expressions at sites of inflammation, and leading to excessive induction of inflammatory mediators which may causing the development of intestinal damage(61).

In this study, colon tissues exposed to acetic acid produced an over expression of iNOS and COX-2messenger as compared with the control negative one, these results were supported by findings of (62, 63).

Concerning to inducible nitric oxide synthase (iNOS), it is an enzyme usually expressed during inflammatory reactions(64). Meanwhile, synthesis of large amounts of nitric oxide (NO) content is demonstrated in acute or chronic inflammation, and it produced largely when iNOS expression is increased.

Overall, elevated of iNOS expression may be an indicator of inflammation progression(10).

Enhancement of COX-2 (inducible cyclooxygenase enzyme) expressions from inflamed colon tissues play an integral role in the pathogenesis of ulcerative colitis (65, 66). Moreover, excessive over expressions of COX-2 lead to prostaglandin (PGE) releasing (67), and ROS production which cause further cell injury (65).

Histopathological finding go side by side with the obtained macroscopic damage score, oxidative and anti-oxidative biomarkers in addition to molecular gene expressions, these data were in harmony with those obtained by(68).

Concerning to Mesalazine, it considered positive drug control which inhibit oxidative stress and ROS releasing from inflamed colon tissue. Moreover, its antioxidant (31) anti-inflammatory effects (69)reduced tissue damage and modulated mortality rate (12.5%).

The current data declared that, gastric intubation of *Lactobacillus* on acetic acid induced colitis showed marked decrease in DAI in comparison with control positive group. This improvement of DAI could be owed to either increase body weigh by increasing energy efficiency (70) or decrease bloody diarrhea and these results were confirmed by decrease mortality rate percent (25%). On the other hand, *Lactobacillus* supplementation revealed significant decline in macroscopic damage score which was confirmed by reduce colon weight associated with significant enhancement of colon length as contrasted with control positive group (71).

The damage score was ameliorated due to the ability of *Lactobacillus* to modulate inflammatory response of colon tissue through interferes with innate immune system and adaptive immunity. Therefore, it enhances anti-inflammatory cytokine production (72). Others attributed the reduction of inflammation by *Lactobacillus* to bacteriocins secretions which act as broad spectrum antimicrobial substances and protect against secondary infections, Consequently decreasing the duration of diarrhea (73). In the other hand, *Lactobacillus* may changepH of the gut flora which leading to reduce inflammatory state of colon(74).

At the same time, lipid peroxidation and antioxidant biomarkers evaluate ability of *Lactobacillus* to reduce ROS and free radicals from inflamed colon (75). In this study, supplementation of *Lactobacillus* revealed an improvement of antioxidant biomarkers (GSH and CAT) and decreased in oxidative stress parameters (MDA and NO) as matched with control positive group, these facts were confirmed by result of (76). Multiple experimental studies demonstrated the antioxidative activities of *Lactobacillus* by secreting enzymatic and non enzymatic anti-oxidant substance and promoting its release from the inflamed colon tissue (77).

Moreover, *Lactobacillus* enhance the production of particular antioxidant biomolecules, for example, exopolysaccharides (EPSs) which probably useful for elimination of oxidative stress from intestine (78). Consequently decrease lipid peroxidation (MDA) and NO marker from inflamed colon tissue. Finally, it exhibited metal chelating activities which get together with the pathogenesis of most chronic diseases(79). These results were confirmed by significant decrease in iNOS andCOX-2 expression in *Lactobacillus* supplemented group as matched with control positive group, these data are in harmony with those obtained by(80).

Consistent with this mechanism, histopathological findings of *Lactobacillus* group were showed moderate colitis. This finding agrees with (12). Therefore, *Lactobacillus* had many therapeutic benefits and was used as vehicles for treatment of gastrointestinal diseases (81)

In this experiment, *Spirulina* supplementation played great role in relieving the incidence of induced colitis. Significant enhancement in DAI and macroscopic damage score were recorded in *Spirulina* group as compared with control positive group, this effect could be explained by the high protein content, amino acids, vitamins (vitamin B complex) and folic acid which induce an increase in nutri-

tional value of this algae and promote weight gain (27).

Similarly, a significant decline in bloody diarrhea was found in *Spirulina* group reach to (0%) may be due to mucopolysachharids content which makes building blocks of colon cell membranes and accelerates healing of colon tissue (30) These results were confirmed by decreased mortality rate to 25% and histopathological findings. Furthermore, gastric intubation of *Spirulina* in acetic acid induced colitis showed a marked decline in colon weight with significant increase in colon length as contrasted with control positive group. This improvement could be referred to ability of *Spirulina* to manage the inflammatory conditions and oxidative damages (82). Phycocyanin present in *Spirulina* considers anti-inflammatory ingredients of it, which decrease production of intracellular ROS and histamine from mast cell, inhibit inflammatory cell infiltration specially neutrophil and reduced edema index in the induced inflammation(83, 84). Besides that, *Spirulina* has excellent antioxidative properties and preservative effects to structural integrity of colon tissue(85). In present study, *Spirulina* supplementation showed improvement in CAT and GSH and significant decrease in NO and MDA in comparison with control positive group. These results confirmed by molecular RNA expression of iNOS and COX-2 which showed significant decrease in contrast with control positive group. The antioxidant anti-inflammatory effects of *Spirulina* could be attributed to its content of phycocyanin and β-carotene which have capability to scavenge free radicals and ROS from inflamed colon tissue (86).

In the same way, *Spirulina* decreased inflammatory mediators and inflammatory cytokines (IL-1β, IL-6, and IL-12) releasing from inflamed colon (87). Therefore, it plays a fundamental role in UC improvement. Moreover, histopathological finding of *Spirulina* showed mild colitis, and this result supported by finding of (32).

Noteworthy, the combination of *Spirulina* and *Lactobacillus* in acetic acid induced colitis afforded a higher protection and more effectiveness than each one alone, this result was confirmed by recording the lowest mortality rate (12.5%), molecular expressions and histopathological findings similar to positive drug control (Mesalazine), that owed to an improvement growth performance and health condition (88). *Spirulina* not only modulate inflammatory response of acetic acid induced colitis, but also it consider as importance nutritional point for *Lactobacillus* due to rich source of protein content, amino acids, vitamins etc which could be needed to nourishment of *Lactobacillus* and improve intestinal colonization (89, 90). Over all, *Spirulina* has growth promoting effect on *Lactobacillus* to reduce the inflammatory effect of ulcerative colitis.

Conclusion

In conclusion, the protective effect of *Lactobacillus* and /or *Spirulina* against experimental colitis in rats could be directly attributed to scavenging ROS, inhibiting lipid peroxidation and suppressing NO releasing. Furthermore, *Lactobacillus* and *Spirulina* have synergistic protective effect on colon tissue and could be used in combination to ameliorate ulcerative colitis.

Conflict of interest

The authors declare that they have no conflict of interest.

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