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The Syk Inhibitor Fostamatinib Decreases the Severity of Colonic Mucosal Damage in a Rodent Model of Colitis

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Abstract

Background and aims: Inflammatory bowel disease is a chronic inflammatory disease of the gastrointestinal system. In some cases, current medications used for inflammatory bowel disease may not be enough for remission, creating a need for more potent and reliable medications. There is no study showing the efficacy of fostamatinib, with proven effects on some inflammatory diseases, on ulcerative colitis. In our study we planned to research the efficacy of fostamatinib, a spleen tyrosine kinase inhibitor, on acetic acid-induced colitis.

Methods: The study included 28 male Sprague-Dawley rats, randomly divided into control group, fostamatinib group, colitis group and fostamatinib + colitis group, each containing seven rats. Colitis induction was performed with 4% acetic acid. Colonic inflammation was assessed with disease activity index, macroscopic and histological damage scores, colonic myeloperoxidase, malondialdehyde and superoxide dismutase activity, and tumour necrosis factor alpha [TNFα], CD3, Syk, and phospho-Syk expression.

Results: There was a significant difference between the colitis and control groups in terms of all parameters. The disease activity index, macroscopic and microscopic damage scores, immunohistochemical TNFα, CD3, Syk, and phospho-Syk expression, and tissue myeloperoxidase activity were found to be significantly lower in the colitis + fostamatinib group compared with the colitis group. There was no significant difference between the two groups in terms of myeloperoxidase and malondialdehyde activity.

Conclusions: Fostamatinib reduced the inflammatory damage in the experimental colitis. This effect may be due to suppression of TNFα, T-lymphocytes, and neutrophils in colonic mucosa via suppression of Syk. Fostamatinib may be an appropriate treatment alternative for ulcerative colitis. Further clinical studies are required to support this.

Keywords: Acetic acid-induced colitis; fostamatinib; rats; spleen tyrosine kinase

1. Introduction

Ulcerative colitis [UC] is a chronic, relapsing inflammatory disease, mainly affecting the gastrointestinal system. In spite of notable developments in recent years, the aetiology of UC has still not been fully explained.[1] It is thought that a defective immune response in the gastrointestinal system due to the effect of environmental risk factors in genetically susceptible individuals is effective in the aetiology of the disease.[2] The defective immune response in UC causes uncontrolled inflammation in the gastrointestinal system, increased permeability of the epithelial barrier and, as a result, penetration of luminal antigens. Luminal antigens that pass the mucosal barrier increase inflammation progressively and as a result give rise to the chronicity.[3,4] The inflammation that occurs disrupts the mucosal integrity and this leads to macroscopic superficial ulcers that progress continuously from distally to proximally.[4]

Currently mesalazine, corticosteroids, immunosuppressives and biological agents are included in the medical treatment options for UC.[5] When the side effect profiles and situations with insufficient response to current treatments are considered in some cases, the need for more potent and more reliable medications is clear.[5,6]

During the inflammatory response, in many cells led by macrophages, some intracellular signal molecules are activated. Among these molecules, the tyrosine kinase family is mentioned as one of the major effective molecules.[7] It was shown that tyrosine kinase inhibitor tyrphostin AG 126 limits the development of colitis in rats with phosphorylation of tyrosine residues.[8] Sakanoue *et al.* demonstrated that protein tyrosine kinase activity in the colonic mucosa increased in UC patients.[9] In a patient with chronic active Crohn's disease [CD], long-standing remission of CD was achieved with the small-molecule receptor tyrosine inhibitor imatinib therapy.[10] It was found that tyrosine kinase-2 gene polymorphisms are associated with UC and CD.[11]

Spleen tyrosine kinase [Syk] is a key protein of the immunoreceptor signalling pathway in immune and inflammatory responses and is a non-receptor tryosine kinase molecule.[12] Syk, contained in neutrophils, T lymphocytes, NK cells, macrophages, dendritic cells, and intestinal epithelial cells, is expressed broadly in haematopoietic and non-haematopoietic cells playing an active role in the inflammatory response.[13] In these cells, Syk plays a role in signal communication of events such as cell adhesion, innate pathogen identification, antibody-mediated cellular cytotoxicity, and inflammatory response, and is also effective in T lymphocyte activation, proliferation and differentiation.[14,15] Syk has been shown to play a pivotal role in macrophage-mediated natural immunity through toll-like receptors. Syk binds to toll-like receptor-4 and allows for phosphorylation of tyrosine residuals. Later it communicates stimulation signals down the pathway, and releases pro-inflammatory cytokines through the nuclear factor kappa beta [NFκβ].[16] Also, β-glucans that are the first antigen of intestinal flora, especially fungi, encountering the innate immunity stimulate dentritic cells via Dectin-1 receptors and the Syk pathway, resulting in production of pro-inflammatory cytokines. β-glucans increase the expression of Dectin-1 and Syk in human peripheral mononuclear cells in CD more than control subjects.[17] However, there is not any study investigating Syk expression in intestinal tissue of inflammatory bowel disease [IBD] patients. It has been shown that many natural compounds, like quercetin, piceatannol, *Rhodomyrtus tomentosa*, and plant extracts, inhibit Syk and suppress inflammation.[18,19,20] It was shown that colitis attenuates in Syk-/- knockout mice, and Syk inhibition attenuates the colitis in Syk-wild mice, but Syk expression level was not evaluated in this study.[21]

Syk phosporylation has a place in the pathogenesis of many inflammatory and autoimmune diseases. Syk plays a role in a variety of inflammatory diseases like rheumatoid arthritis [RA], systemic lupus erythematosus, allergic asthma and rhinitis, idiopathic thrombocytopenic purpura [ITP], and intestinal ischaemic reperfusion damage. Some animal studies have shown that Syk inhibition may be an alternative strategy for the treatment of these inflammatory diseases.[22] Some Syk inhibitors have been shown to regress the symptoms of inflammation: R406 [tamatinib] improved ITP and RA; R788 [fostamatinib], a prodrug of R406, improved ischaemic reperfusion damage, systemic lupus erythematosus, ITP, and RA; R112 improved allergic rhinitis; and R343 improved allergic asthma.[23,24,25,26,27] In addition to these inflammatory events, Syk has been proposed to play a critical role in psoriasis, atherosclerosis, and inflammatory bowel disease as macrophage-mediated inflammatory diseases.[28] In the literature there is no study evaluating the effect of Syk inhibitor, fostamatinib, on UC as a chronic inflammatory disease. Our study planned to research the therapeutic effect of fostamatinib in rats with colitis induced by acetic acid.

2. Materials and Methods

2.1. Ethical statement

The study design and the experimental procedures were approved by the Local Ethical Committe for Animal Studies of Trakya University [Edirne, Turkey] [protocol no: TÜHDYEK-2013/40, approval no: 2013.04.01, date: 5 May 2013].

2.2. Drugs and chemicals

The Syk inhibitor used in our study, fostamatinib [R788], was obtained from Rigel Pharmaceuticals [San Francisco, CA]. For myeloperoxidase [MPO] activity, the rat ELISA kit for MPO [art. no: SEA601Ra; Cloud-Clone Corp., Houston, TX, USA] was used. Kits used in immunohistochemical study were rabbit polyclonal primary antibody for tumour necrosis factor alpha [TNFα] [cat. no: NBP1-67821; NOVUS Biologicals, Littleton, CO, USA], rabbit polyclonal primary antibody for CD3 [cat. no: 250588; Abbiotec, San Diego, CA, USA, dilution 1/100], rabbit anti-rat Syk [N-19] [1:100; Santa Cruz, CA, USA], and rabbit anti-rat phospho-Syk [p-Syk], active component of Syk [phospho Y323] [1:100; Abcam Inc]. The acetic acid solution for colitis induction was obtained from Sigma-Aldrich [St Louis, MO, USA], xylazine for analgesia and sedation was obtained from Bayar [Rompun®, Istanbul, Turkey], and ketamine was obtained from Pfizer [Ketalar®, Istanbul, Turkey].

2.3. Animals

The study used 28 Sprague-Dawley male rats weighing 170–350 g, obtained from the Experimental Animal Research Facility of Trakya University [Edirne, Turkey]. The rats were kept in the laboratory for 4 days before the experiment, for adaptation. The animals were housed in cages, in an environment with no specific pathogens and optimum laboratory conditions until the end of the experiment. The cages contained a maximum of seven rats. The animals were fed standard feed and water orally and 12-h periods of night-day were provided. Room temperature was 23°C and humidity was held at 50–55%. All experimental procedures in the study were in accordance with the principles of the animal experiments local ethics committee.

2.4. Experimental design

The rats were randomly divided into four groups with seven animals in each group [control, fostamatinib-treated, colitis, and colitis + fostamatinib-treated groups]. All rats were starved for 24 h before

the day of the experiment. Only unlimited water was provided to the animals. On the day of the procedure, immediately before the procedure the animals in all groups were weighed. Later all rats had a 6Fr polyethylene enteral feeding tube inserted and intestinal cleaning was completed with 20 ml 0.9% saline using a 20-ml injector. The control group were given 2 ml water by oral gavage and by rectal catheter. The fostamatinib-treated group was given 30 mg/kg/day fostamatinib dissolved in 2 ml of water by oral gavage and 2 ml of water by rectal catheter.[24] The colitis group was given 2 ml water by oral gavage and 2 ml 4% acetic acid solution by rectal catheter. The colitis + fostamatinib-treated group was given 30 mg/kg/day fostamatinib dissolved in 2 ml of water by oral gavage and 2 ml 4% acetic acid solution by rectal catheter. On Days 1–6, the control and colitis groups were given 2 ml water by oral gavage whereas the fostamatinib-treated and colitis + fostamatinib-treated groups were given 30 mg/kg/day fostamatinib dissolved in 2 ml of water by oral gavage. All rats were given standard feed and tapwater on Days 0–6. For gavage and rectal administration, a 6 Fr polyethylene enteral feeding tube was used. All rats were starved for 24 h before sacrifice. Immediately before sacrifice, the rats in all groups were weighed and diarrhoea and rectal haemorrhage were assessed. The rats were sacrificed under anaesthesia on the Day 7 and the colons were removed. Sedation for the rats for gavage and intrarectal administrations and for sacrifice was provided by xylazine [10 mg/kg] and ketamine [100 mg/kg].

2.5. Induction of experimental colitis

Colitis induction was performed in accordance with the method of Mascolo *et al.*[29] The rats in the colitis and colitis + fostamatinib-treated groups were starved for 1 day and given ketamine/xylazine sedation before the procedure. Fifteen min after intestinal cleaning, the anus was entered with a 6 Fr polyethylene enteral feeding tube which was advanced to 8 cm. In the Trendelenburg position, 2 ml of 4% acetic acid was injected. To prevent the acetic acid from coming out, the rats were pressed rectally and held upside down for 60 s. Later the acetic acid was removed from the catheter using the injector, and the colon was washed with 5 ml 0.9% saline.

2.6. Collection of tissue samples

Under deep sedation, the rats were sacrificed by cervical dislocation and the abdomen was incised with a midline incision. The distal 12–13 cm of the colon was quickly removed and opened longitudinally on the anti-mesenteric side. The interior of the intestine was gently washed with 0.9% saline. After macroscopic images were obtained, the material was divided into two parts: half was placed in 10% formalin for macroscopic, histological and immunohistochemical assessment. The other half was stored at -80°C for biochemical analysis.

2.7. Evaluation of disease activity

To evaluate the disease activity, a modification of the disease activity index [DAI] proposed by Cooper *et al.*[30] was used [Table 1]. All rats were weighed before colitis induction and before sacrifice to determine weight loss score. Stool consistency and rectal haemorrhage situations were assessed to calculate stool and haemorrhage scores just before sacrifice. The scores from these three parameters were separately summed to calculate DAI.

2.8. Macroscopic scoring

Macroscopic assessment of the colon was performed by two independent histologists. To calculate the macroscopic damage score, a modification of the criteria reported by McCafferty *et al.*[31] was used [Table 2].

2.9. Histopathological examination

Tissue samples, after 24 h fixation in phosphate-buffered saline containing 10% formalin solution, underwent routine histological procedures to obtain paraffin blocks. The paraffin blocks were serially cut to 5-µm sections and stained with haematoxylin-eosin [H&E]. The histological changes in the colon were assessed and photographed by two independent histologists using an Olympus BX51 [Tokyo, Japan] light microscope. Histopathological scoring was performed according to the criteria described by Obermeier *et al.*[32] [Table 3].

2.10. Assessment of MPO activity

The tissue samples from the colon were homogenised with 1.15% potassium chloride. Half of the obtained homogenate was studied for malondialdehyde [MDA]. The other half was centrifuged for 1 h at 5 000 revolutions per min [rpm] [+4°C]. MPO activity measured from the obtained supernatant using rat the ELISA MPO kit [Cloud-Clone Corp. Houston, TX, USA] according to the manufacturer's protocol. The results are expressed as ng/mg protein.

2.11. Assessment of SOD activity

For superoxide dismutase [SOD] analysis, the supernatants were first centrifuged at 3 000 rpm for 10 min with chloroform and ethanol. The upper portion was used for analysis. SOD analysis was performed according to the method reported by Sun *et al.*[33] In

Table 1. Criteria for the scoring of disease activity index.[30]

Weight loss %	Stool consistency	Rectal bleeding	Score
< 1%	Normal	Absence	0
1–5%	-	-	1
5–10%	Loose stools	Presence	2
10–15%	-	-	3
> 15%	Diarrhoea	Gross bleeding	4

Table 2. Criteria for macroscopic scoring of colonic damage.[31]

Feature	Score
Ulceration	
Normal appearance	0
Focal hyperaemia, no ulcers	1
Ulceration without hyperaemia or bowel wall thickening	2
Ulceration with inflammation at one site	3
Two or more sites with ulceration and inflammation	4
Major sites of damage extending > 1 cm along the length of the colon	5
When an area of damage extended > 2 cm along length of colon, the score of was increased by 1 for each additional cm of involvement	6–10
Adhesions	
No adhesions	0
Minor adhesions [colon can be easily separated from other tissue]	1
Major adhesions	2
Diarrhoea	
No	0
Yes	1
Thickness	
Maximal bowel wall thickness [x], in millimetres	x
	Total score

Table 3. Criteria for histopathological scoring of colonic damage.[32]

Epithelium [E]	Score
Normal morphology	0
Loss of goblet cells	1
Loss of goblet cells in large areas	2
Loss of crypts	3
Loss of crypts in large areas	4
Infiltration [I]	
No infiltrate	0
Infiltrate around crypt basis	1
Infiltrate reaching to lamina muscularis mucosae	2
Extensive infiltration reaching the lamina muscularis mucosae and thickening of the mucosa with abundant oedema	3
Infiltration of the lamina submucosa	4
The total histological score	[E+I]

the protein analysis of homogenate and supernatant, the method of Lowry *et al.*[34] was used. The obtained SOD activity levels are adapted to protein levels. The results are given as U/g protein.

2.12. Determination of lipid peroxidation

Lipid peroxidation was determined using the procedure described by Yoshoiko *et al.*,[35] in which MDA, an end-product of fatty acid peroxidation, in 0.5 ml colonic tissue homogenates reacted with 2 ml of thiobarbituric acid [TBA, Sigma Chemical, St Louis, MO] reagent containing 0.375% thiobarbituric acid, 15% trichloroacetic acid, and 0.25 N hydrochloric acid. Samples were boiled for 15 min, then cooled and centrifuged. The colour change in supernatants was measured spectrophotometrically with a maximum absorbance at a wavelength 532 nm. The results were expressed as µmol per gram of protein in samples.

2.13. Immunohistochemical study

Five-micrometre paraffin tissue sections were deparaffinised by using xylene and rehydrated in ethanol. Then the sections were boiled in citrate buffer [10 mM; pH 6.0; Thermo Scientific/Lab Vision, Fremont, CA, USA] for 10 min for antigen retrieval. After washing with phosphate buffer saline, the sections were immersed in 3% hydrogen peroxide in distilled water for 10 min to inhibit endogenous peroxidase activity. The non-specific binding of antibodies was blocked by incubation with a blocking serum [Thermo Scientific/Lab Vision] at room temperature for 5 min. The sections were incubated with rabbit polyclonal primary antibodies; anti-CD3 [Abbiotech, San Diago, CA, USA, dilution 1/100], anti-TNFα [cat. no: NBP1-67821; NOVUS Biologicals, Littleton, CO, USA], rabbit anti-rat Syk [1:100; Santa Cruz Biotechnology, Santa Cruz, CA, USA], rabbit anti-rat phospho-Syk [phospho Y323] [1:100; Abcam Inc.] at room temperature for 60 min. The sections were washed three times with phosphate buffer saline and incubated with biotinylated secondary antibody [Ultra Vision Detection System-HRP kit, Thermo Scientific/Lab Vision] at room temperature for 10 min. Three-amino-9-ethylcarbazole [AEC, Substrate System, Thermo Scientific/Lab Vision] was used as a chromogen. The sections were then counterstained with haematoxylin. The tissue sections were examined under light microscopy [400x], and CD3-positive, TNFα-positive,Syk, and p-Syk-positive cells were counted in random 100 high-power fields using an Olympus BX51 light microscope [Tokyo, Japan] in each sample. Number of positive cells per mm^2 was recorded for each group in CD3 and TNFα staining. Semiquantitative histo-score method [H-score] was used in assessing the expression of Syk and p-Syk.[36] The staining intensity was graded as: 0, no staining, 1, weak; 2, moderate; 3, strong; and 4, very strong.

2.1.4 Statistical considerations

All statistical analysis were made by using IBM Statistical Package for the Social Sciences [SPSS] Statistics for Windows software [Version 20.0. Armonk, NY: IBM Corp.]. The non-parametric continuous variables were interpreted with KruskalWallis for multiple comparisons, then the MannWhitney U test for two-groups comparisons followed by manual adjustment for *p*-value by the Bonferroni method. The significant ^{p}-value was assumed as $^{<}$ 0.05. The results were presented as mean ± standard deviation.

3. Results

3.1. Disease activity index

The groups were primarily assessed in terms of stool consistency, rectal haemorrhage, and weight loss scores. The stool consistency score of the colitis group [4.0 ± 0] was significantly higher than the control [0] and fostamatinib [0] groups [$p < 0.001$]. The stool consistency score in the colitis + fostamatinib [0] group was significantly lower than the colitis group [$p < 0.001$], but there was no significant difference compared with the control and fostamatinib groups. The stool consistency scores of control and fostamatinib groups were similar. Although rectal haemorrhage was only observed in the colitis group, there was no significant difference observed between the groups [rectal haemorrhage score for the colitis group 2.5 ± 2.1; other groups 0] [Figure 1A].

Whereas the control group gained weight during the experiment, the other groups lost weight [Table 4]. While the weight loss score in the colitis group [2.13 ± 0.6] was higher by a significant degree compared with the control group [$p = 0.001$], there was no significant difference compared to the fostamatinib group [1.83 ± 0.4]. The weight loss score in the colitis + fostamatinib group [1.86 ± 0.7] was similar to the colitis and fostamatinib groups but was higher by a significant degree compared with the control group [$p = 0.001$]. The weight loss score in the fostamatinib group was higher by a significant degree compared with the control group [$p = 0.001$] [Figure 1A].

When the groups are assessed in terms of DAI, the score in the colitis group [9.0 ± 2.0] was higher by a significant degree than the control [0] and fostamatinib groups [1.83 ± 0.4] [$p = 0.001$]. The disease activity score in the colitis + fostamatinib group [1.86 ± 0.7] was lower by a significant degree than the colitis group [$p = 0.001$] and higher by a significant degree than the score of the control group [$p = 0.001$]. Whereas there was no significant difference between the disease activity scores of the fostamatinib and colitis + fostamatinib groups, the scores in the fostamatinib groups were significantly higher than the control group [$p = 0.001$] [Figure 1B].

3.2. Macroscopic evaluation

Macroscopic evaluation of the colon tissues found severe colonic mucosal ulceration, oedema and haemorrhage in the colitis group compared with the control group. The inflammation in the colitis + fostmatinib group was clearly less compared with the colitis group [Figure 2A]. The macroscopic damage score of the colitis group [10 ± 1.1] was found to be higher by a significant degree compared with the control [0] and fostamatinib groups [0.17 ± 0.1] [$p = 0.001$, $p = 0.002$, respectively]. The macroscopic damage score in the colitis + fostamatinib group [6.5 ± 0.5] was higher by a significant degree than the control and fostamatinib

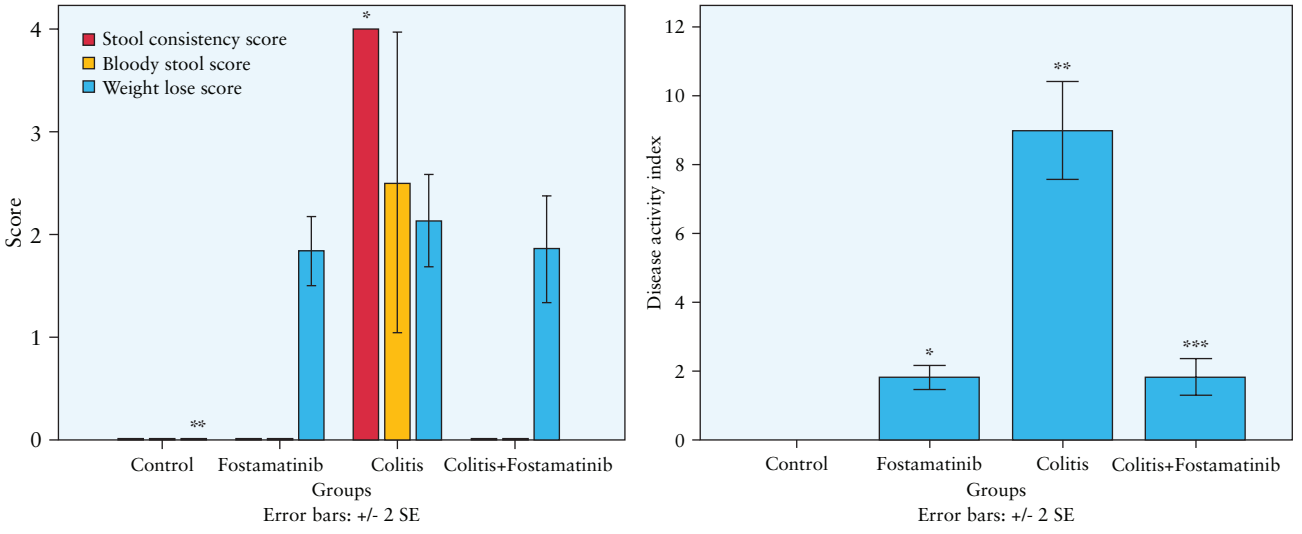

Figure 1. Stool consistency, rectal bleeding, weight loss and Disease Activity Index analysis in the experimental groups. Data were analysed by KruskallWallis followed by MannWhitney U test. Values are expressed as mean ± standard error of mean; $n = 7$ in each group. SE, standard error. A. $^{*}p < 0.001$ compared with control, fostamatinib, and colitis + fostamatinib groups; $^{**}p = 0.001$ compared with fostamatinib, colitis, and colitis + fostamatinib groups. B. $^{*}p = 0.001$ compared with control and colitis groups; $^{**}p = 0.001$ compared with control and colitis+fostamatinib groups; $^{***}p = 0.001$ compared with control group.

Table 4. Changes in body weight in the experimental groups.

Groups	Baseline weight [g]	Final weight [g]	Change in weight [%]
Control[a]	288 ± 16.5	298 ± 16.7	3.48 ± 0.55
Fostamatinib	305 ± 27.5	282 ± 23.2	-7.27 ± 1.80
Colitis	199 ± 1 6.9	186 ± 19.4	-6.68 ± 2.45
Colitis + fostamatinib	277 ± 17.3	260 ± 26.6	-6.67 ± 4.06

The results are shown as the mean ± standard deviation.

[a]$p < 0.05$ compared with fostamatinib, colitis, and colitis + fostamatinib groups.

groups [$p = 0.001$, $p = 0.002$, respectively] and lower than the colitis group [$p = 0.001$]. The score in the fostamatinib group was significantly higher than the control group [$p = 0.007$] [Figure 2B].

3.3. Histopathological evaluation

Histopathological assessment of colon tissues found severe inflammatory cell infiltration in the colitis group compared with the control group. There was a clear regression observed in inflammation in the colitis + fostamatinib group compared with the colitis group [Figure 3A]. The microscopic damage score in the colitis group [6.5 ± 0.5] was higher by a significant degree compared with the control [0] and fostamatinib groups [0.4 ± 0.1] [$p = 0.001$, $p = 0.002$, respectively]. The microscopic damage score in the colitis + fostamatinib group [4.1 ± 0.9] was significantly higher than that in the control and fostamatinib groups [$p = 0.002$, $p = 0.003$, respectively] and lower than the colitis group [$p = 0.001$]. The score in the fostamatinib group was significantly higher than that in the control group [$p = 0.002$] [Figure 3B].

3.4. Biochemical measurements

When the groups were assessed in terms of biochemical parameters, the MPO activity in colonic tissue samples in the colitis group [71.5 ± 3.6 ng/mg] was found to be higher by a significant degree compared with the control [33.4 ± 12.3 ng/mg] and fostamatinib groups [47.6 ± 4.5 ng/mg] [$p = 0.004$]. The MPO activity in the colitis + fostamatinib group [53.9 ± 5.2 ng/mg] was significantly lower than the activity in the colitis group [$p = 0.003$], with no significant difference compared with the control and fostamatinib groups. There was no significant difference between the control and fostamatinib groups in terms of MPO activity [Figure 4A].

The SOD activity in colonic tissue samples from the colitis group [27.6 ± 3.1 U/g] was significantly lower than that in the control group [45.3 ± 5.4 U/g] [$p = 0.004$], but there was no significant difference compared with the fostamatinib group [30.7 ± 2.5 U/g]. The SOD activity in the colitis + fostamatinib group [26.7 ± 5.2 U/g] was similar to the colitis and fostamatinib groups and was significantly lower than the control group [$p = 0.003$]. The SOD activity in the control group was higher by a significant degree compared with the fostmatinib group [$p = 0.004$] [Figure 4B].

The MDA levels in colonic tissue samples from the colitis group [12.3 ± 1.3 µmol/g] were higher by a significant degree compared with the control group [9.8 ± 0.5 µmol/g] [$p = 0.004$], but were not significantly different from the fostmatinib group [11.8 ± 1.9 µmol/g]. The MDA levels in the colitis + fostamatinib group [12.1 ± 1.3 µmol/g] were similar to the colitis and fostamatinib groups, and were found to be higher by a significant degree compared with the control group [$p = 0.003$]. There was no significant difference between the control and fostamatinib groups in terms of MDA levels [Figure 4C].

3.5. Immunohistochemical staining

In the immunohistochemical assessment of colon tissues, the CD3 and TNFα expression were compared in the groups. Although clear CD3 expression was observed in the colitis group compared with the control group, there was a reduction in CD3 expression in the colitis +f ostamatinib group compared with the colitis group [Figure 5A]. The CD3+ T lymphocyte counts per square millimetre in the colitis group [121.6 ± 30.6/mm^2] were higher by a significant degree than the control [19.2 ± 14.6/mm^2] and fostamatinib groups [16 ± 12.2/mm^2] [$p < 0.001$]. In the colitis + fostamatinib group, the CD3+ T lymphocyte count [41.6 ± 21.7/mm^2]

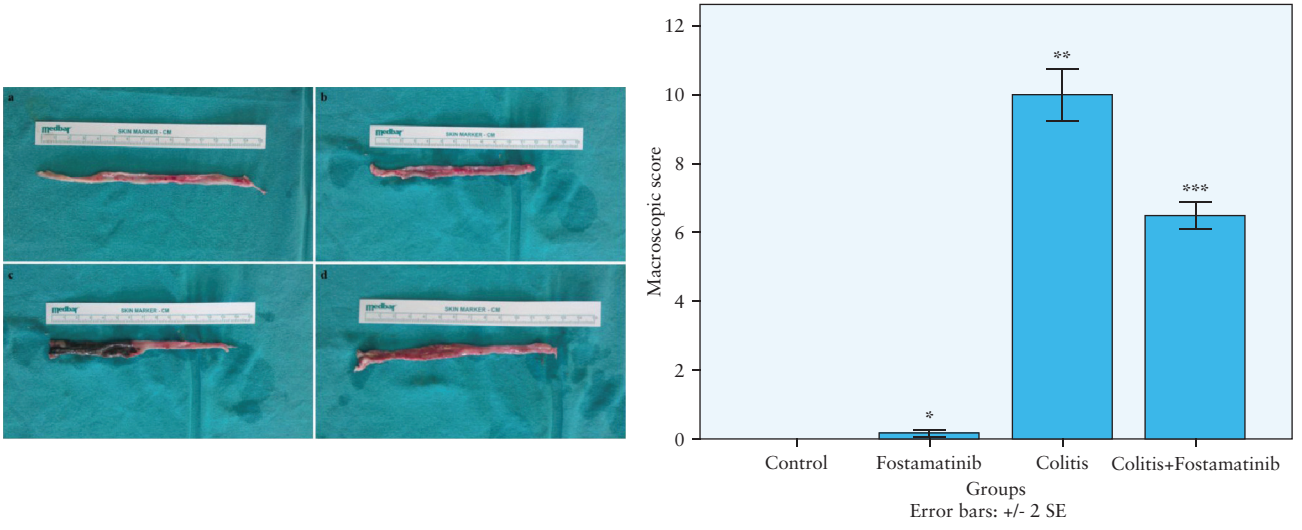

Figure 2. The effect of fostamatinib on macroscopic damage score. A. Macroscopic presentation of colonic tissue specimens of the experimental groups. Normal colonic mucosa in control group [a]; and fostamatinib-treated [30 mg/kg four times a day] group [b]; colonic mucosa showed macroscopically ulcerative, oedematous ,and haemorrhagic epitelium in untreated acetic acid-induced colitis group [c]; significantly improved colonic mucosa in fostamatinib-treated [30 mg/kg four times a day] acetic acid-induced colitis group [d]. B. Data were analysed by KruskallWallis followed by MannWhitney U test. Values are expressed as mean ± standard error of mean; $n = 7$ in each group. SE, standard error. *$p = 0.007$ compared with control group, $p = 0.002$ compared with colitis and colitis+fostamatinib groups; **$p = 0.001$ compared with control and colitis+fostamatinib groups; ***$p < 0.001$ compared with control group.

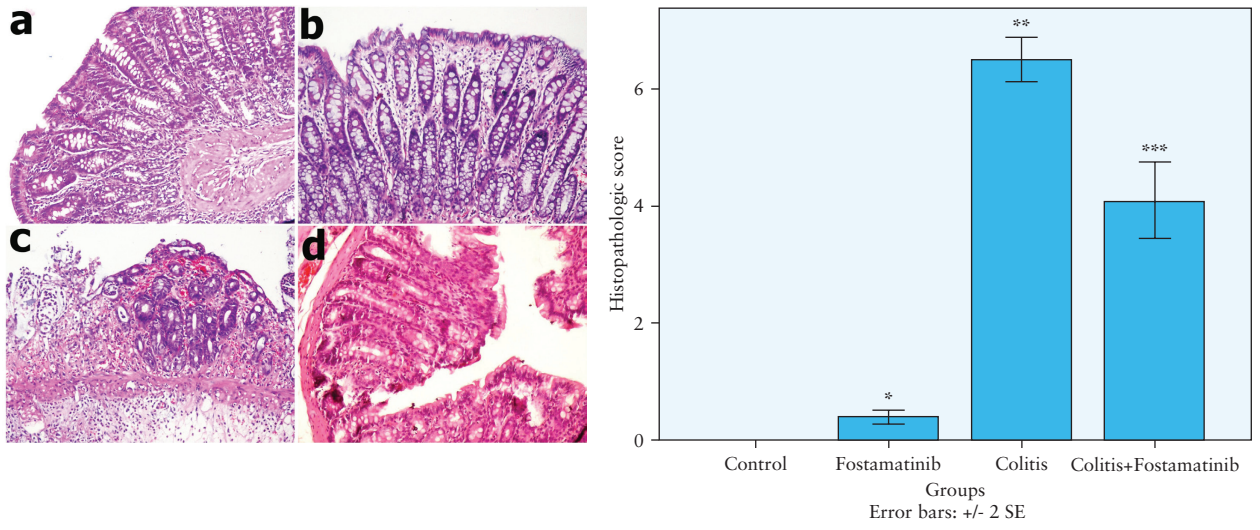

Figure 3. The effect of fostamatinib on microscopic damage score. A. Histological presentation of colonic tissue specimens of the experimental groups. The histology of colon tissues was normal in appearance in the control group [a] and fostamatinib-treated [30 mg/kg four times daily] group [b]; colonic mucosa showed microscopically severe ulceration, oedema, haemorrhage, and severe inflammation in untreated acetic acid-induced colitis group [c]; colonic tissue consisting of moderate ulceration and inflammation in fostamatinib-treated [30 mg/kg four times daily] acetic acid-induced colitis group [d]. [H&E; Magnifications: 200x]. B. Data were analysed by KruskallWallis followed by MannWhitney U test. Values are expressed as mean ± standard error of mean; $n = 7$ in each group. SE, standard error. *$p = 0.002$ compared with control and colitis groups, $p = 0.003$ compared with colitis+fostamatinib groups; **$p = 0.001$ compared with control and colitis+fostamatinib groups; ***$p = 0.002$ compared with control group.

was significantly higher than the control and fostamatinib groups [$p < 0.001$] and lower than the colitis group [$p < 0.001$]. There was no significant difference between the fostamatinib and control groups [Figure 5B].

When tissue samples were compared in terms of TNFα expression, the TNFα+ cell count in the colitis group was clearly greater than the control group. There was a reduction observed in TNFα expression in the colitis + fostamatinib group compared with the colitis group [Figure 6A]. The TNFα+ cell count per square millimetre in the colitis group [9.16 ± 2.4/mm^2] was higher by a significant degree compared with the control [3.04 ± 1.2/mm^2] and fostamatinib groups [1.32 ± 1.3/mm^2] [$p < 0.001$]. The TNFα+ cell count in the colitis + fostamatinib group [5.24 ± 1.4/mm^2] was higher by a significant degree compared with control and fostamatinib groups [$p < 0.001$] and lower than the colitis group [$p < 0.001$]. The TNFα expression in the fostamatinib group was significantly lower than the control group [$p < 0.001$] [Figure 6B].

The expression of Syk and p-Syk in colonic tissue was stronger in the colitis group whereas it was weak in the control group.

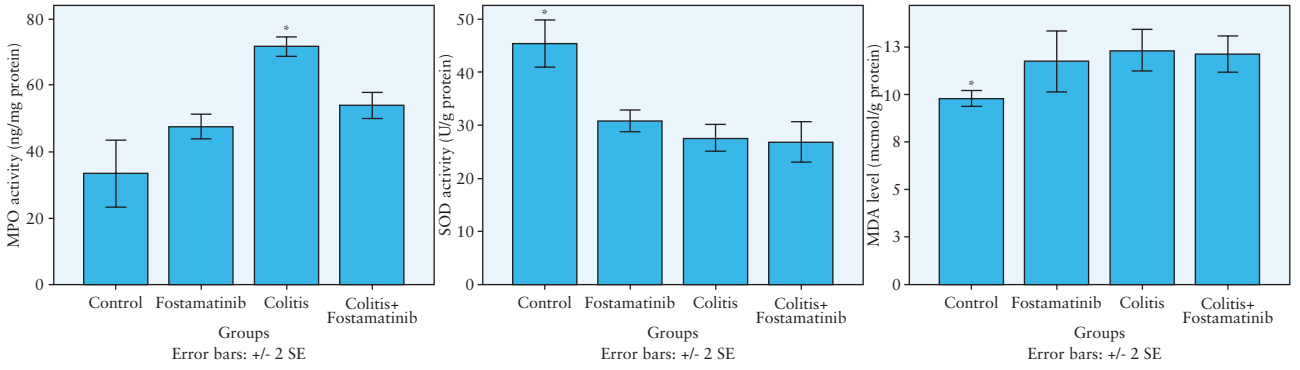

Figure 4. The effect of fostamatinib on colonic tissue myeloperoxidase, superoxide dismutase activity, and malondialdehyde level in acetic acid-induced colitis in rats. Data were analysed by KruskalWallis followed by MannWhitney U test. Values are expressed as mean ± standard error of mean; $n = 7$ in each group. MPO, myeloperoxidase; SOD, superoxide dismutase; MDA, malondialdehyde; SE, standard error. A. *$p = 0.004$ compared with control and fostamatinib groups; $p = 0.003$ compared with colitis+fostamatinib group. B. *$p = 0.004$ compared with fostamatinib and colitis groups; $p = 0.003$ compared with colitis+fostamatinib group. C. *$p = 0.004$ compared with colitis group; $p = 0.003$ compared with colitis + fostamatinib group.

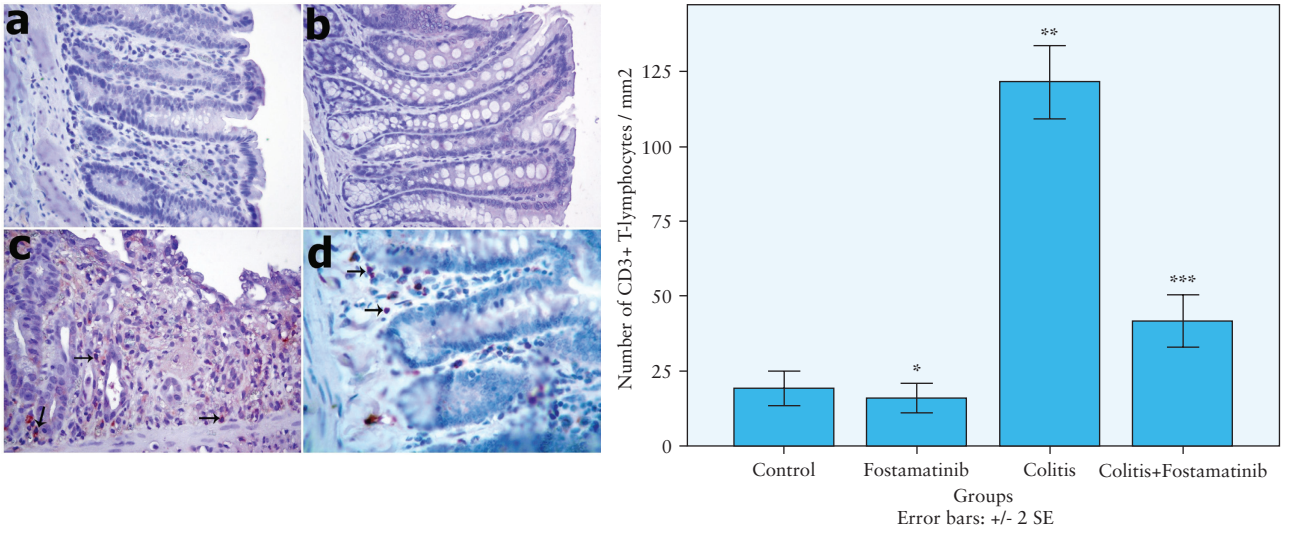

Figure 5. A. Immunohistochemical photomicrographs of CD3-positive staining T lymphocytes in colonic mucosa of the experimental groups. Control [a]; fostamatinib-treated [30 mg/kg four times daily] [b]; acetic acid-induced colitis [c]; acetic acid induced-colitis + fostamatinib-treated [30 mg/kg four times daily] [d]. [Arrows: CD3-positive cells; immunoperoxidase, haematoxylin counterstain; magnifications: 200x]. B. Expression of CD3 immunohistochemical staining section in colonic mucosa of each experimental group [number of positively staining T lymphocytes per mm^2]. Data were analysed by KruskalWallis followed by MannWhitney U test. Values are expressed as mean ± standard error of mean; $n = 7$ in each group. SE, standard error. *$p < 0.001$ compared with colitis and colitis+fostamatinib groups; **$p < 0.001$ compared with control and colitis+fostamatinib groups; ***$p < 0.001$ compared with control group.

There was a weaker expression of Syk and p-Syk in the colitis + fostamatinib group compared with the colitis group [Figure 7A and 8 A]. The intensity of Syk+ cells in the colitis group [H-score: 3.65 ± 0.67] was significantly higher compared with the control group [H-score: 1.55 ± 0.61] [$p = 0.0001$]. The intensity of Syk+ cells in the colitis + fostamatinib group [H-score: 1.90 ± 0.64] was significantly lower than the colitis group [$p = 0.0001$]. The intensity of Syk expression in the colitis + fostamatinib group was similar to the control group [$p = 0.082$] [Figure 7B]. Similarly, the intensity of p-Syk+ cells in the colitis group [H-score: 3.60 ± 0.50] was significantly higher compared with the control group [H-score: 1.40 ± 0.60] [$p = 0.0001$], and the intensity of p-Syk+ cells in the colitis + fostamatinib group [H-score: 1.95 ± 0.69] was lower than the colitis group [$p = 0.0001$]. The intensity of p-Syk+ cells in the colitis + fostamatinib group was significantly higher than the control group [$p = 0.01$] [Figure 8B].

4. Discussion

Our study shows that fostamatinib, a Syk inhibitor, affected inflammatory cells and proinflammatory cytokines and provided histological and morphological healing to a clear degree in acetic acid-induced colitis. This is the first study to investigate the effect of Syk inhibition by fostamatinib on acetic acid-induced colitis. Our findings show that Syk expression increases in experimental colitis and inhibition of Syk reduces the expression of Syk in the colonic tissue and, as a result, fostamatinib has a protective effect on colitis. This supports that idea that the Syk signal pathway is active during the inflammatory process created during colitis induction. Fostamatinib may contribute to treatment of UC with its anti-inflammatory effect.

The protective effect of Syk inhibition has been shown by many studies for different autoimmune, allergic, and chronic inflammatory diseases.[12,21,23] This effect is probably realised through the

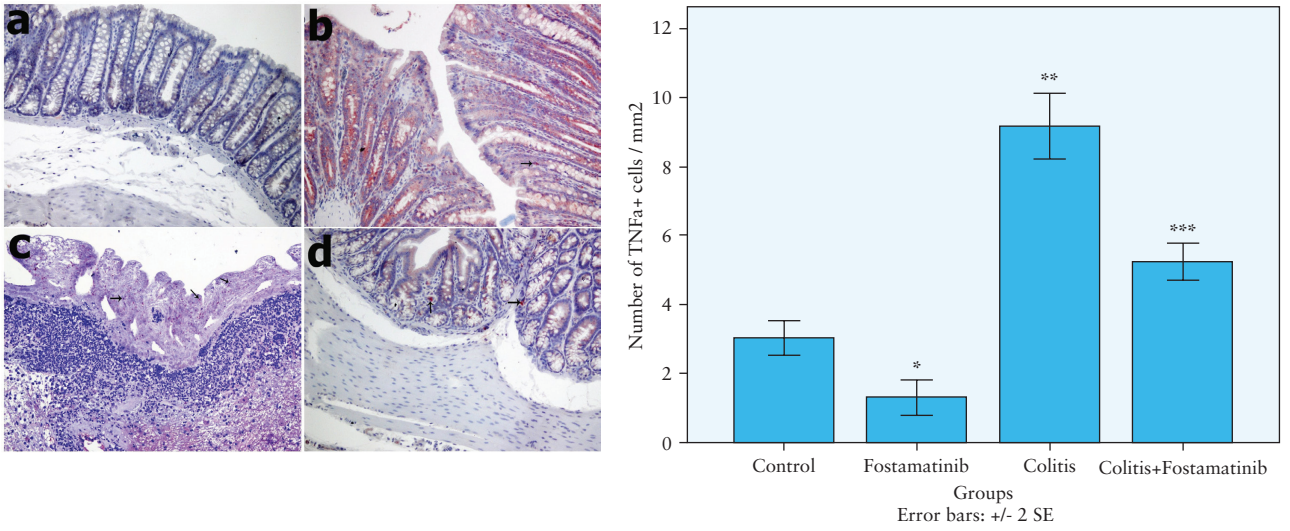

Figure 6. A. Immunohistochemical photomicrographs of TNF-α-positive staining cells in colonic mucosa of the experimental groups. Control [a]; fostamatinib-treated [30 mg/kg four times daily] [b]; acetic acid-induced colitis [c]; acetic acid-induced colitis + fostamatinib-treated [30 mg/kg four times daily] [d]. [Arrows: TNFα-positive cells; immunoperoxidase, haematoxylin counterstain; magnifications: 200x]. B. Expression of TNFα immunohistochemical staining section in colonic mucosa of each experimental group [number of positively staining cells per mm^2]. Data were analysed by KruskallWallis followed by MannWhitney U test. Values are expressed as mean ± standard error of mean; ^{n} = 7 in each group. TNF-α, tumour necrosis factor-alpha; SE, standard error. *p < 0.001 compared with control, colitis, and colitis + fostamatinib groups; **p < 0.001 compared with control and colitis + fostamatinib groups; ***p < 0.001 compared with control group.

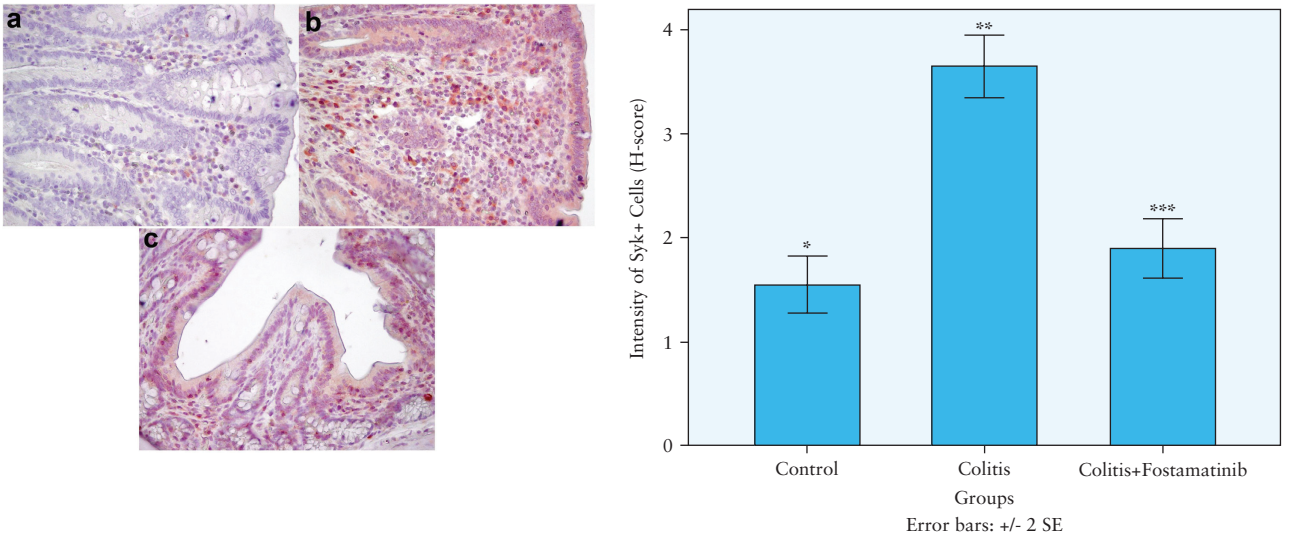

Figure 7. A. Immunohistochemical photomicrographs of Syk-positive staining cells in colonic mucosa of the experimental groups. Weak expression of Syk in control group [a]; strong expression of Syk in acetic acid-induced colitis group [b]; weak-moderate expression of Syk in acetic acid-induced colitis + fostamatinib-treated [30 mg/kg four times daily] group [c]. [Immunoperoxidase, haematoxylin counterstain; magnifications: 400x]. B. Intensity of Syk immunohistochemical staining section in colonic mucosa of each experimental group [H-score]. Data were analysed by KruskallWallis followed by MannWhitney U test. Values are expressed as mean ± standard error of mean; n = 7 in each group. Syk, spleen tyrosine kinase; SE, standard error. *p < 0.0001 compared with colitis group; **p < 0.0001 compared with colitis + fostamatinib groups; ***p = 0.082 compared with control group.

Syk-dependent receptors on haemapoietic and normal tissue cells.[37] Dendritic cells and T lymphocytes have a very effective role in the pathogenesis of UC.[38] The effect of Syk inhibition on inflammation in colitis is partially formed by its effect on haematopoietic cells.[37]

Previous studies have reported a tendency for acetic acid-induced colitis to spontaneously heal, but reported it to be most utilised murine model because it is very quick, easy, and reproducible.[39,40] Chemical insult results in diffuse colitis with barrier dysfunction. At the histological level, it induces diffuse ulceration along with inflammatory infiltration and the distortion of crypt architecture in distal colonic tissue. The acetic acid-induced colitis model is more suitable for the demonstration of early pathogenic mechanisms in IBD as an acute colitis.[40] Histopathological and clinical characteristics are greatly similar to UC in humans.[39] The acetic acid-induced colitis model is characterised by oxidative stres, mucosal polymorphonuclear cell infiltration, TNFα and NFκβ and their involved inflammatory cascade activation, and finally macroscopic and histopathological changes.[41,42] However, the mucosal events in this

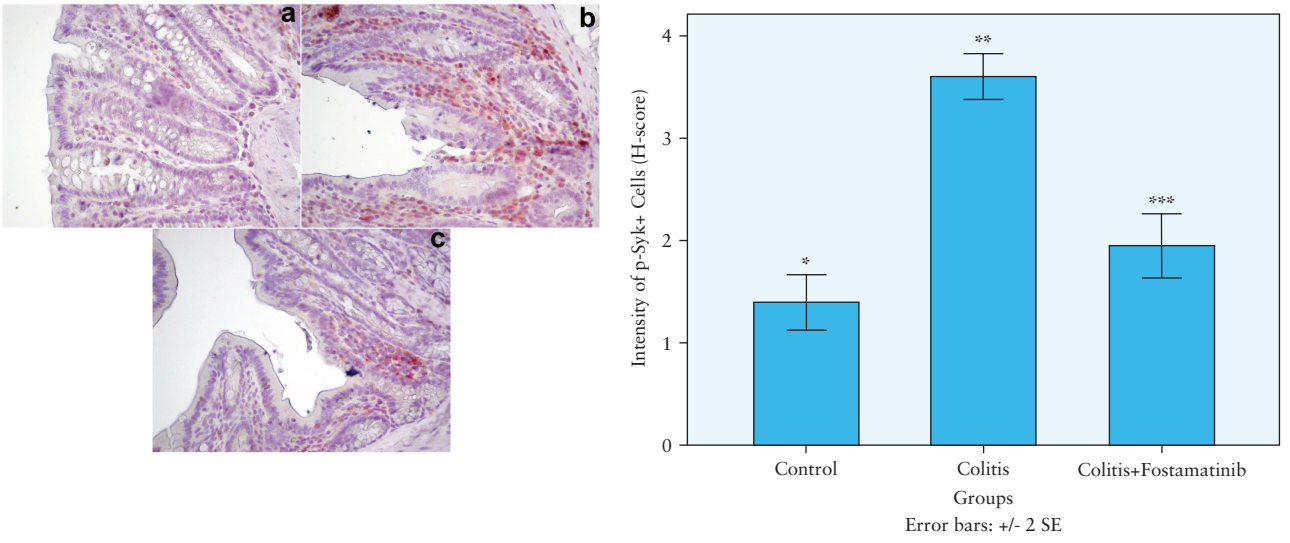

Figure 8. A. Immunohistochemical photomicrographs of p-Syk-positive staining cells in colonic mucosa of the experimental groups. Weak expression of p-Syk in control group [a]; strong expression of p-Syk in acetic acid-induced colitis group [b]; weak-moderate expression of p-Syk in acetic acid-induced colitis + fostamatinib-treated [30 mg/kg four times daily] group [c]. [Immunoperoxidase, haematoxylin counterstain; magnifications: 400x]. B. Intensity of p-Syk immunohistochemical staining section in colonic mucosa of each experimental group [H-score]. Data were analysed by KruskallWallis followed by MannWhitney U test. Values are expressed as mean ± standard error of mean; $n = 7$ in each group. p-Syk, phosphorylated spleen tyrosine kinase, SE, standard error. $^{*}p < 0.0001$ compared with colitis group; $^{**}p < 0.0001$ compared with colitis + fostamatinib group; $^{***}p = 0.01$ compared with control group.

model are not representative of the immunological background in IBD; because of that, the results must be evaluated with caution.[40] To assess the severity of inflammatory events in experimental colitis models, frequently morphological damage, histopathological changes, and DAI calculated according to bloody stool, diarrhoea and weight loss scores are used.[41] Previous studies have reported that rectal haemorrhage and weight loss, histological and morphological mucosal thickening in the colon, inflammatory cell infiltration in the mucosa and submucosa, oedema, ulceration, and loss of crypts and goblet cells have been observed in colitis models induced with acetic acid diarrhoea that is clinically similar to UC.[29,43,44] In our study, 4% acetic acid was used for colitis induction. It was observed that the colitis model was successfully formed histologically, biochemically, and immunohistochemically on the evaluation. Diarrhoea and haemorrhage were only observed in the colitis group, whereas weight loss was observed in all groups apart from the control group. Although fostamatinib prevented diarrhoea and haemorrhage, the rats administered this medication had as much weight loss as the rats with colitis. Weight loss is thought to be due to the primary effect of the medication. When evaluated as a whole, the very high DAI in those with colitis clearly regressed in the rats with colitis given fostamatinib treatment. It can be said that the treatment was clinically effective. However, as one of the components assessed in the disease activity evaluations was weight loss, which was high in the groups given fostamatinib, the DAI scores did not regress to the level of the control group. If we consider the high DAI in the groups administered fostamatinib as a side effect of medication, it is more correct to assess it differently from the weight loss associated with colitis.

In the colitis group, severe inflammation in the form of macroscopic ulcers in the mucosa, oedema and occasional haemorrhagic foci, and microscopically neutrophil infiltration, oedema, and ulcers were observed. In subjects with colitis induction given fostamatinib, the mucosa was significantly protected from morphological and histological inflammation. However, inflammation was not completely resolved. Similar to results of clinical markers of the study, it was seen that medication increased the inflammation by a certain amount regardless of colitis induction. A poor correlation was found between microscopic findings and endoscopic and clinical pictures for UC, with histologically active findings observed in the most patients with UC in clinical and endoscopic remission; microscopically active disease may persist in an endoscopically inactive disease.[45] Clinical improvement was more dramatic than macroscopic and histological healing in our results. The discordance between clinical and histological features in our results may mirror with this poor correlation in UC patients.

There are studies supporting the idea that in the inflammatory response, changes in oxidative parameters such as MPO, MDA, nitrite, and glutation [GSH] occur due to oxidative stress.[46] In an acute inflammation, severe neutrophil infiltration occurs. MPO is a good marker for acute inflammatory events reflecting neutrophil activity biochemically.[43] MPO is the most essential weapon of the natural immune system, forming the first line of defence, and increases in direct relation to the severity of inflammation in the inflammatory response.[43] After intrarectal acetic acid administration, neutrophil infiltration is induced as a means of acute inflammation. It is reported that MPO levels increased secondary to oxidative damage and neutrophil infiltration in colitis.[43,47] In our study, a clear increase was observed in MPO levels in the colitis group which regressed notably with fostamatinib treatment. Studies have shown that Syk inhibition reduces neutrophil infiltration without affecting its bacterial cleaning effect.[48] At the same time, Syk affects actin polymerisation, and Syk inhibition is reported to slow rapid actin polymerisation and reduce chemotaxis of the neutrophils. In conclusion, it may be considered that Syk reduces neutrophil infiltration by this mechanism.[49]

In the inflammatory process, the first line of defence against oxidative stress in the organism is formed by GSH. During colitis induction, the free oxygen radicals released activated NFκβ and increased proinflammatory cytokines. Macrophages and leukocytes that reach the area of inflammation increase the amount of reactive oxygen

radicals. In this way inflammation is made more severe.[50] Free oxygen radicals are caught by SOD and converted to hydrogen peroxide. Catalase and glutation peroxidase enzymes convert hydrogen peroxide to water and render it ineffective. During inflammation there is a reduction of SOD activity and GSH amounts due to consumption. It is expected in acetic acid-induced colitis that there should be a reduction in SOD and GSH relative to severity of inflammation.[51] The free oxygen radicals released as a result of oxidative stress disrupt cell membrane stability and cause cell death by lipid peroxidation. The triggered lipid peroxidation disrupts the integrity of the intestinal mucosal barrier and causes activation of inflammatory mediators.[52] Another parameter used to assess severity of inflammation in our study, MDA, is one of the markers of oxidative damage as it is the final product of lipid peroxidation. In parallel to the increase in reactive oxygen radicals, MDA levels increase.[46] In groups administered fostamatinib, the changes in MDA and SOD levels were similar to the colitis group. These findings support the idea that fostamatinib does not have a protective effect against oxidative damage. Besides, fostamatinib alone causes similar rates of oxidative damage as colitis. This finding brings to mind that the clinically, macroscopically, and microscopically emerging tableau is the result of oxidative damage considered as a side effect of the medication. The reduction in MPO levels related to medication may be explained as a reduction in neutrophil infiltration rather than oxidative stress. Fostamatinib may not have antioxidant effects but may have anti-inflammatory effects. This may be carried out due to the effect on proinflammatory cytokines and inflammatory cells in the Syk signal pathway.[37]

To better understand the molecular mechanism of the effect of fostamatinib, TNFα, CD3, Syk, and p-Syk expressions were evaluated immunohistochemically in the colonic tissue in the study. TNFα is a strong cytokine functioning in signal transmission mechanism, that is effective in the migration of neutrophils to the site of inflammation. Though produced by mast cells, CD4+ lymphocytes, neutrophils, and NK cells, it is basically released from macrophages. The TNFα levels in serum and tissues are used to essentially evaluate the severity of inflammation.[47] CD3 is a marker for T lymphocytes and after being produced in the thymus is expressed by T lymphocytes at all stages. It is known that TNFα and CD3 expression play an important role in UC and their expression levels increase in acute inflammation.[46] It is not known whether Syk is involved in the inflammation of IBD. Intestinal epithelial cells respond to stimulation with β-glucans, fungal antigen of intestinal flora, via the Syk pathway in healthy subjects, and Syk expression increases in these cells.[53] There are not any data about the expression level of Syk in intestinal tissue of IBD patients. In our study, increased expression of Syk and p-Syk, active form of Syk, in colonic tissue of the rats in the acetic acid-induced colitis group showed that the Syk pathway is actively involved in the inflammation of acute colitis. Attenuation of the colitis along with the reduction of the Syk and p-Syk expression in the colon as a result of Syk inhibition with fostamatinib give rise the idea that the Syk pathway may be a part of the pathogenesis of IBD, and fostamatinib may be useful in the treatment of IBD. Many inflammatory signalling pathways intersect at NFκβ and it plays a key role in the expression of many proinflammatory cytokines and TNFα. When the Syk signal pathway active in acute inflammation is inhibited by fostamatinib, the level of TNFα expression decreases. There are studies supporting the idea that the inflammatory effect of the Syk signal pathway is carried out through NFκβ.[37] In the colitis group, the clear increase in TNFα and CD3 expression shows that colitis was induced properly. In the colitis group treated with fostamatinib, the expression levels were significantly reduced compared with the colitis group, supporting the idea that the medication has clear anti-inflammatory effect. The reduction in TNFα and CD3 expression to lower levels than the control group by fostamatinib alone, may give rise to the thought that the drug shows intrinsic inflammatory effect,as we identified clinically and biochemically, at the level of oxidative damage, not at cellular or cytokine levels.

In conclusion, the obtained results show that fostamatinib has potent anti-inflammatory activity against the variety of pathological changes due to intrarectal administration of acetic acid and healed colitis; however, it does not have an antioxidant mechanism. This study supports that fostamatinib may have a potential role in treatment options of UC. Further studies are required to investigate the effect of the medication on UC in humans and reveal the effects on fibrosis and immunological mechanisms of the disease with more suitable murine models.

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Conflict of Interest

The authors have not declared any conflicts of interest.

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References

1. Liang J, Sha SM, Wu KC. Role of the intestinal microbiota and fecal transplantation in inflammatory bowel diseases. *J Dig Dis* 2014;**15**:641–6.
2. Davies JM, Abreu MT. The innate immune system and inflammatory bowel disease. *Scand J Gastroenterol* 2015;**50**:24–33.
3. Goll R, van Beelen Granlund A. Intestinal barrier homeostasis in inflammatory bowel disease. *Scand J Gastroenterol* 2015;**50**:3–12.
4. Fiocchi C. Inflammatory bowel disease: etiology and pathogenesis. *Gastroenterology* 1998;**115**:182–205.
5. Bryant RV, Brain O, Travis SP. Conventional drug therapy for inflammatory bowel disease. *Scand J Gastroenterol* 2015;**50**:90–112.
6. Park SC, Jeen YT. Current and emerging biologics for ulcerative colitis. *Gut Liver* 2015;**9**:18–27.
7. Lin YC, Huang DY, Chu CL, Lin WW. Anti-inflammatory actions of Syk inhibitors in macrophages involve non-specific inhibition of toll-like receptors-mediated JNK signaling pathway. *Mol Immunol* 2010;**47**:1569–78.
8. Cuzzocrea S, McDonald MC, Mazzon E, *et al.* The tyrosine kinase inhibitor tyrphostin AG 126 reduced the development of colitis in the rat. *Lab Invest* 2000;**80**:1439–53.

9. Sakanoue Y, Hatada T, Horai T, Okamoto T, Kusunoki M, Utsunomiya J. Increased protein tyrosine kinase activity of the colonic mucosa in ulcerative colitis. *Scand J Gastroenterol* 1992;**27**:686–90.
10. Magro F, Costa C. Long-standing remission of Crohn's disease under imatinib therapy in a patient with Crohn's disease. *Inflamm Bowel Dis* 2006;**12**:1087–9.
11. Can G, Tezel A, Gurkan H, *et al.* Tyrosine kinase-2 gene polymorphisms are associated with ulcerative colitis and Crohn's disease in Turkish population. *Clin Res Hepatol Gastroenterol* 2015, 2 Mar. doi: 10.1016/j.clinre.2015.01.005. [Epub ahead of print.]
12. Kaur M, Singh M, Silakari O. Inhibitors of switch kinase 'spleen tyrosine kinase' in inflammation and immune-mediated disorders: a review. *Eur J Med Chem* 2013;**67**:434–46.
13. Duta F, Ulanova M, Seidel D, *et al.* Differential expression of spleen tyrosine kinase Syk isoforms in tissues: Effects of the microbial flora. *Histochem Cell Biol* 2006;**126**:495–505.
14. Sada K, Takano T, Yanagi S, Yamamura H. Structure and function of Syk protein-tyrosine kinase. *J Biochem* 2001;**130**:177–86.
15. Sedlik C, Orbach D, Veron P, *et al.* A critical role for Syk protein tyrosine kinase in Fc receptor-mediated antigen presentation and induction of dendritic cell maturation. *J Immunol* 2003;**170**:846–52.
16. Hamerman JA, Tchao NK, Lowell CA, Lanier LL. Enhanced toll-like receptor responses in the absence of signaling adaptor DAP12. *Nat Immunol* 2005;**6**:579–86.
17. Baram L, Cohen-Kedar S, Spektor L, Elad H, Guzner-Gur H, Dotan I. Differential stimulation of peripheral blood mononuclear cells in Crohn's disease by fungal glycans. *J Gastroenterol Hepatol* 2014;**29**:1976–84.
18. Jeong D, Yang WS, Yang Y, *et al.* In vitro and in vivo anti-inflammatory effect of Rhodomyrtus tomentosa methanol extract. *J Ethnopharmacol* 2013;**146**:205–13.
19. Endale M, Park SC, Kim S, *et al.* Quercetin disrupts tyrosine-phosphorylated phosphatidylinositol 3-kinase and myeloid differentiation factor-88 association, and inhibits MAPK/AP-1 and IKK/NF-kappaB-induced inflammatory mediators production in RAW 264.7 cells. *Immunobiology* 2013;**218**:1452–67.
20. Ashikawa K, Majumdar S, Banerjee S, Bharti AC, Shishodia S, Aggarwal BB. Piceatannol inhibits TNF-induced NF-kappaB activation and NF-kappaB-mediated gene expression through suppression of IkappaBalpha kinase and p65 phosphorylation. *J Immunol* 2002;**169**:6490–7.
21. Mencarelli A, Renga B, Distrutti E, Cipriani S, Fiorucci S. Essential role of DNAX Adaptor Protein 12 (DAP12) and spleen tyrosine kinase (Syk) in inflammation-driven immune dysfunction in rodent model of colitis. *Gastroenterol* 2011;140(5):S–499.
22. Wong BR, Grossbard EB, Payan DG, Masuda ES. Targeting Syk as a treatment for allergic and autoimmune disorders. *Expert Opin Investig Drugs* 2004;**13**:743–62.
23. Podolanczuk A, Lazarus AH, Crow AR, Grossbard E, Bussel JB. Of mice and men: an open-label pilot study for treatment of immune thrombocytopenic purpura by an inhibitor of Syk. *Blood* 2009;**113**:3154–60.
24. Pine PR, Chang B, Schoettler N, *et al.* Inflammation and bone erosion are suppressed in models of rheumatoid arthritis following treatment with a novel Syk inhibitor. *Clin Immunol* 2007;**124**:244–57.
25. Pamuk ON, Lapchak PH, Rani P, Pine P, Dalle Lucca JJ, Tsokos GC. Spleen tyrosine kinase inhibition prevents tissue damage after ischemia-reperfusion. *Am J Physiol Gastrointest Liver Physiol* 2010;**299**:G391–9.
26. Meltzer EO, Berkowitz RB, Grossbard EB. An intranasal Syk-kinase inhibitor [R112] improves the symptoms of seasonal allergic rhinitis in a park environment. *J Allergy Clin Immunol* 2005;**115**:791–6.
27. Bahjat FR, Pine PR, Reitsma A, *et al.* An orally bioavailable spleen tyrosine kinase inhibitor delays disease progression and prolongs survival in murine lupus. *Arthritis Rheum* 2008;**58**:1433–44.
28. Pamuk ON, Tsokos GC. Spleen tyrosine kinase inhibition in the treatment of autoimmune, allergic and autoinflammatory diseases. *Arthritis Res Ther* 2010;**12**:222.
29. Mascolo N, Izzo AA, Autore G, Maiello FM, Di Carlo G, Capasso F. Acetic acid-induced colitis in normal and essential fatty acid deficient rats. *J Pharmacol Exp Ther* 1995;**272**:469–75.
30. Cooper HS, Murthy SN, Shah RS, Sedergran DJ. Clinicopathologic study of dextran sulfate sodium experimental murine colitis. *Lab Invest* 1993;**69**:238–49.
31. McCafferty DM, Sharkey KA, Wallace JL. Beneficial effects of local or systemic lidocaine in experimental colitis. *Am J Physiol* 1994;**266**:G560–7.
32. Obermeier F, Kojouharoff G, Hans W, Scholmerich J, Gross V, Falk W. Interferon-gamma [IFN-gamma]- and tumour necrosis factor [TNF]-induced nitric oxide as toxic effector molecule in chronic dextran sulphate sodium [DSS]-induced colitis in mice. *Clin Exp Immunol* 1999;**116**:238–45.
33. Sun Y, Oberley LW, Li Y. A simple method for clinical assay of superoxide dismutase. *Clin Chem* 1988;**34**:497–500.
34. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951;**193**:265–75.
35. Yoshioka T, Kawada K, Shimada T, Mori M. Lipid peroxidation in maternal and cord blood and protective mechanism against activated-oxygen toxicity in the blood. *Am J Obstet Gynecol* 1979;**135**:372–6.
36. Gatalica Z, Bing Z. Syk tyrosine kinase expression during multistep mammary carcinogenesis. *Croat Med J* 2005;**46**:372–6.
37. Cui S, Bilitewski U. [Anti-inflammatory effect of Syk inhibitor in LPS stimulated macrophages]. *Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi* 2013;**29**:1024–7.
38. Ikeda Y, Akbar F, Matsui H, Onji M. Characterization of antigen-presenting dendritic cells in the peripheral blood and colonic mucosa of patients with ulcerative colitis. *Eur J Gastroenterol Hepatol* 2001;**13**:841–50.
39. Elson CO, Sartor RB, Tennyson GS, Riddell RH. Experimental models of inflammatory bowel disease. *Gastroenterology* 1995;**109**:1344–67.
40. Latella G, Rogler G, Bamias G, *et al.* Results of the 4th scientific workshop of the ECCO [I]: pathophysiology of intestinal fibrosis in IBD. *J Crohns Colitis* 2014;**8**:1147–65.
41. Neurath MF, Fuss I, Pasparakis M, *et al.* Predominant pathogenic role of tumor necrosis factor in experimental colitis in mice. *Eur J Immunol* 1997;**27**:1743–50.
42. Neurath MF, Pettersson S, Meyer zum Buschenfelde KH, Strober W. Local administration of antisense phosphorothioate oligonucleotides to the p65 subunit of NF-kappa B abrogates established experimental colitis in mice. *Nat Med* 1996;**2**:998–1004.
43. Yuan H, Ji WS, Wu KX, Jiao JX, Sun LH, Feng YT. Anti-inflammatory effect of Diammonium Glycyrrhizinate in a rat model of ulcerative colitis. *World J Gastroenterol* 2006;**12**:4578–81.
44. Karaca T, Simsek N, Uslu S, *et al.* The effect of royal jelly on CD3[+], CD5[+], CD45[+] T-cell and CD68[+] cell distribution in the colon of rats with acetic acid-induced colitis. *Allergol Immunopathol [Madr]* 2012;**40**:357–61.
45. Rosenberg L, Nanda KS, Zenlea T, *et al.* Histologic markers of inflammation in patients with ulcerative colitis in clinical remission. *Clin Gastroenterol Hepatol* 2013;**11**:991–6.
46. Podolsky DK. Inflammatory bowel disease. *N Engl J Med* 2002;**347**:417–29.
47. Soyturk M, Saygili SM, Baskin H, *et al.* Effectiveness of Saccharomyces boulardii in a rat model of colitis. *World J Gastroenterol* 2012;**18**:6452–60; discussion p 69.
48. Rosenbloom J, Castro SV, Jimenez SA. Narrative review: fibrotic diseases: cellular and molecular mechanisms and novel therapies. *Ann Intern Med* 2010;**152**:159–66.
49. Kyttaris VC, Tsokos GC. Syk kinase as a treatment target for therapy in autoimmune diseases. *Clin Immunol* 2007;**124**:235–7.
50. Reifen R, Nissenkorn A, Matas Z, Bujanover Y. 5-ASA and lycopene decrease the oxidative stress and inflammation induced by iron in rats with colitis. *J Gastroenterol* 2004;**39**:514–9.
51. Dodda D, Chhajed R, Mishra J. Protective effect of quercetin against acetic acid induced inflammatory bowel disease [IBD] like symptoms in rats: possible morphological and biochemical alterations. *Pharmacol Rep* 2014;**66**:169–73.
52. Verspaget HW, Pena AS, Weterman IT, Lamers CB. Diminished neutrophil function in Crohn's disease and ulcerative colitis identified by decreased oxidative metabolism and low superoxide dismutase content. *Gut* 1988;**29**:223–8.
53. Cohen-Kedar S, Baram L, Elad H, Brazowski E, Guzner-Gur H, Dotan I. Human intestinal epithelial cells respond to beta-glucans via Dectin-1 and Syk. *Eur J Immunol* 2014;**44**:3729–40.