OPEN ACCESS Pharmacologia

ISSN 2044-4648
DOI: 10.5567/pharmacologia.2016.114.124

Research Article
Ameliorative Effect of Ferulic Acid Against Acetic Acid Induced Ulcerative Colitis: Role of HO-1 and Nrf2

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Abstract

Background: Inflammatory Bowel Disease (IBD) is a chronic disease of unknown etiology, which is characterized by chronic and spontaneously relapsing inflammation. **Objective:** To evaluate the effect of ferulic acid on acetic acid-induced IBD in rats. **Materials and Methods:** Ulcerative colitis was induced in male Wistar rats (180-220 g) by intrarectal instillation of 2 mL of 4% (v/v) acetic acid solution. Rats were treated orally with either ferulic acid (10, 20 and 40 mg kg^{-1}, p.o.), prednisolone (2 mg kg^{-1}) or distilled water (10 mg kg^{-1}). Various biochemical, molecular and histological parameters were evaluated. **Results:** Intrarectal administration of 4% acetic acid resulted in significant alteration ($p<0.05$) in ulcer area, serum alkaline phosphatase, serum lactate dehydrogenase and colonic superoxide dismutase (SOD), glutathione (GSH), malondialdehyde (MDA) and myeloperoxidase (MPO) content. Administration of ferulic acid (20 and 40 mg kg^{-1}) significantly ($p<0.05$) ameliorated these acetic acid-induced alterations. There was a significant ($p<0.05$) down-regulation in colonic HO-1 mRNA expression, which was significantly up-regulated ($p<0.05$) by ferulic acid (20 and 40 mg kg^{-1}). The decreased colonic Nfr2 level after acetic acid instillation was increased by ferulic acid (20 and 40 mg kg^{-1}) treatment, which was revealed by immunohistochemical analysis. Histological aberration induced after acetic acid instillation was inhibited by ferulic acid. **Conclusion:** The findings of the present investigation showed that ferulic acid has an anti-inflammatory and anti-oxidant potential to inhibit acetic acid-induced colitis via upregulation in the HO-1 and Nrf-2 expressions.

Key words: Acetic acid-induced colitis, HO-1, Nrf-2, inflammatory bowel disease, ferulic acid, oxidative stress

Received: February 21, 2016 Accepted: March 01, 2016 Published: March 15, 2016

Citation: Amit D. Kandhare, Anagha Patil, Anamika Guru, Anwesha Mukhrjee, Sandipan Sarkar, Antara Sengupta, Hari Mohan Parmar, Amol P. Muthal, Pralhad Wangikar and Subhash L. Bodhankar, 2016. Ameliorative effect of ferulic acid against acetic acid induced ulcerative colitis: Role of HO-1 and Nrf2. Pharmacologia, 7: 114-124.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Crohn's disease and ulcerative colitis are the forms of a continuum of subtypes in Inflammatory Bowel Disease (IBD)[1,2]. The IBD is a chronic, relapsing, immune inflammation disorder of intestinal tract. Although the pathophysiology of IBD is unknown, however impaired immune response in intestinal mucosa due to the influx of Reactive Oxygen Species (ROS) increases the $CD4^+$ T lymphocytes, mast cells, neutrophil and eosinophil count, that orchestrate mucosal inflammation, hemorrhage and development of strictures in the colon[3,4]. Other factors that contribute to the development and maintenance of IBD includes enteric microflora, *Helicobacter pylori* infection, genetic and environmental factors[5,6].

The prevalence of IBD among different races has been debated and various pools of thought exist in this regard. Africans, Americans, South Asians have been reported to possess increased susceptibility to developing IBD as compared to Caucasians. The onset of IBD includes 18-60 years of age with a higher ratio of male to female in Ulcerative Colitis (UC) than Crohn's disease. The incidence of UC is 24.3/100,000/person/years whereas, prevalence is 505/100,000/person/years[7]. Moreover, the incidence of UC in pediatric IBD is 2.8/100,000/person/years, which is increased by 20% in both developed and developing countries[8].

Literature cited with evidence suggests that production of Reactive Oxygen Species (ROS) plays a vital role in tissue injury in IBD patient as well as in animal model too[9,10]. Respiratory burst of infiltrating phagocytic cells leads to elevated Reactive Oxygen Species (ROS) production. It is also reported that activated macrophages responsible for the release of pro-inflammatory cytokines, such as tumor necrosis factor-α, interleukin-1 and interleukin-8, which lead to the production of oxidative mediators, such as ROS lead to tissue damage[11,12].

Acetic acid induced ulcerative colitis is a well-established, highly reproducible animal model, which phenotypically resembles human colon inflammation[13,14]. When acetic acid instilled intrarectally, it causes non-transmural inflammation reflected by elevated neutrophil infiltration into the intestinal tissue, mucosal and submucosal necrosis, vascular dilation, edema and ulceration of submucosa. Acetic acid induced colitis is associated with epithelial damage, which resulting from intracellular acidification of colon that occurred due to the entry of protons that are released after protonation of acetic acid[15].

The current treatment regimen for IBD involve drugs, such as anti-inflammatory (prednisolone, sulphasalazine, 5-aminosalicylic acid), immunomodulatory drugs (azathioprine and mercaptopurine) and antibiotics[16]. However, these agents provide partial relief in patients and its also associated with marked side effects. Thus, development of new therapeutic moieties with low deleterious effect is need of the hour.

Ferulic acid ((E)-3-(4-hydroxy-3-methoxy-phenyl)prop-2-enoic acid), a polyphenol is present abundantly in the vegetables and maize bran[17]. It is an essential component of Chinese medicinal herbs. Ferulic acid shows its potent antioxidant activity by scavenging free radicals, which up-regulats cytoprotective enzymes[18-20]. It has an ability to inhibit the expression of cytotoxic enzymes, such as cyclooxygenase-2[21]. Moreover, various polyphenols and flavonoids have been shown to alleviate chronic inflammation in an experimental model of IBD[22]. Various pharmacological activities of ferulic acid have been well documented by previous researchers, which include anti-oxidants, anti-inflammatory, antidiabetic, antimicrobial, hepatoprotective, anti-cholesterolemic, neuroprotective, anti-carcinogenic, UV protection, radioprotection, etc.[23,24]. However, its potential in inflammatory bowel disease has not been yet evaluated. Hence, the aim of present investigation was to determine the role of ferulic acid in amelioration of acetic acid-induced experimental colitis in laboratory animals.

MATERIALS AND METHODS

Animals: Adult male Wistar rats (180-220 g) were obtained from the National Institute of Biosciences, Pune, India. They were maintained at 24±1°C, with a relative humidity of 45-55% and 12:12 h dark/light cycle. The animals had free access to standard pellet chow (Pranav Agro Industries Ltd., Sangli, India) and water throughout the experimental protocol. All experiments were carried out between 09:00 and 17:00 h. The experimental protocol (CPCSEA/77/2012) was approved by the Institutional Animal Ethics Committee (IAEC) of Poona College of Pharmacy, Pune and performed in accordance with the guidelines of Committee for Control and Supervision of Experimentation on Animals (CPCSEA).

Drugs and chemicals: Ferulic acid was purchased from Sigma Chemical Co. (St Louis, MO, USA). Prednisolone was obtained as a gift sample from Symed Pharmaceutical Pvt. Ltd., Hyderabad. Total RNA Extraction kit and One-step Reverse Transcription-Polymerase Chain Reaction (RT-PCR) kit was purchased from MP Biomedicals India Private Limited, India.

Induction of colitis and drug treatment schedule: Colitis was induced according to the previously described procedure[14,16]. Rats were randomly divided into following groups consisting of 6 rats as follows:

- **Normal animals (N):** Received 1 mL of distilled water (10 mg kg^{-1}, p.o.) for 12 days
- **Acetic acid control animals (AA control):** Received 2 mL of 4% acetic acid solution (once, intrarectally) and distilled water (10 mg kg^{-1}, p.o.) for 12 days
- **Prednisolone (2 mg kg^{-1}) treated animals (Pred 2):** Received prednisolone (2 mg kg^{-1}, p.o., for 5 days) and acetic acid (2 mL of 4% solution, once, intrarectally). Prednisolone and acetic acid treatment were started on the same day
- **Ferulic acid (10 mg kg^{-1}) treated animals (FA 10):** Received 7 days pretreatment with ferulic acid (10 mg kg^{-1}, p.o.) and 2 mL of 4% acetic acid solution, intrarectally on the 8th day. Drug treatment was continued until 12th day
- **Ferulic acid (20 mg kg^{-1}) treated animals (FA 20):** Received 7 days pretreatment with ferulic acid (20 mg kg^{-1}, p.o.) and 2 mL of 4% acetic acid solution, intrarectally on the 8th day. Drug treatment was continued until 12th day
- **Ferulic acid (40 mg kg^{-1}) treated animals (FA 40):** Received 7 days pretreatment with ferulic acid (40 mg kg^{-1}, p.o.) and 2 mL of 4% acetic acid solution, intrarectally on the 8th day. Drug treatment was continued till 12th day

Ferulic acid was administered to rats in three different dosages (10, 20 and 40 mg kg^{-1}) for 12 days. On the 13th day, rats were sequentially anesthetized with anesthetic ether for about 30-40 sec. Blood was withdrawn by retro-orbital puncture. Each blood sample was collected into separate vials for determination of serum parameters. After blood collection, the animals were sacrificed by cervical dislocation, the colon was excised and frozen immediately in liquid nitrogen and stored at -80°C for further biochemical and histopathological examination.

Assessment of colonic damage, ulcer area and ulcer index: The severity of colitis was evaluated by an independent observer, who was blinded to the treatment. For each animal, the distal 10 cm portion of the colon was removed and cut longitudinally, slightly cleaned in physiological saline to remove fecal residues and weighed. Macroscopic inflammation scores were assigned based on clinical features of the colon. The presence of adhesions (score 0-10) and stool consistency (score 0-4) were evaluated according to the previously reported method[14,16]. The evaluation of ulcer area and ulcer index were performed according to the previously reported method[25,26].

Determination of serum parameters: Serum activities of LDH and ALP were measured using ultraviolet-visible spectrophotometer (JASCO-V-530, JASCO Corp., Tokyo, Japan) and commercially available reagent kits (Accurex Biomedical Pvt. Ltd. and Pathozyme Diagnostics, India).

Biochemical assays: For colon homogenization, tissue segments were mixed with 0.1 M phosphate buffer and homogenized on ice bath for 60 sec at 10000 rpm in a homogenizer (Remi equipment Pvt. Ltd., Remi motors Ltd., Mumbai, India). Supernatant of tissue homogenates were employed to estimate superoxide dismutase (SOD), reduced glutathione (GSH) and lipid peroxidation (MDA content) as previously described[27-31]. The colonic myeloperoxidase (MPO) assay was determined according to previously described method[32,33].

RNA extraction and RT-PCR analysis: The levels of mRNA were analyzed in colon tissue using RT-PCR as described previously[34,35]. Single-stranded cDNA was synthesized from 5 µg of total cellular RNA using reverse transcriptase kit (MP Biomedicals India Private Limited, India) as described previously[36-38]. The primer sequence for heme oxygenase 1 (HO-1) and β-actin are presented in the supplementary file. Amplification of β-actin served as a control for sample loading and integrity. The PCR products were detected by electrophoresis on a 1.5% agarose gel containing ethidium bromide. The size of amplicon was confirmed using a 100 bp ladder as a standard size marker. The amplicon was visualized and images were captured using a gel documentation system (Alpha Innotech Inc., San Leandro, CA, USA). Expression of gene was assessed by generating densitometry data for band intensities in different sets of experiments and was generated by analyzing the gel images on the Image J software (Version 1.33, Wayne Rasband, NIH, Bethesda, MD, USA) semi-quantitatively. The band intensities were compared with constitutively expressed β-actin. The intensity of mRNAs were standardized against that of the β-actin mRNA from each sample and the results were expressed as PCR-product/β-actin mRNA ratio.

Immunohistochemical detection of Nrf2 protein: Samples of the colon were fixed in 10% buffered formalin for 24 h, embedded in paraffin and sectioned. The sections were deparaffinized in xylene and rehydrated before analysis, immersed in 3% H_2O_2 to quench endogenous peroxidase activity and microwaved in 10 mM sodium citrate (pH 6.0) for 15 min for antigen retrieval. Five percent normal goat serum was applied to eliminate nonspecific background staining. The sections were then incubated overnight with primary rabbit anti-human Nrf2-specific antibody (1:100; Santa Cruz Biotechnology, Santa Cruz, CA, USA) at 4°C in a high humidity chamber. Then HRP-conjugated goat antirabbit IgG (Santa Cruz Technology) was added to the sections and incubated for 20 min at room temperature. The sections were stained with DAB solution, counterstained with hematoxylin, washed for 1 h with PBS, air-dried and covered with mounting medium. A known sample from a patient with breast cancer was used as a positive control and negative control slides were processed with PBS in place of primary antibody. All samples were evaluated blindly by the same pathologist. The results of immunohistochemical staining were analyzed using Image-Pro Plus (IPP) 5.0.

Histological examination: Freshly excised colon of one animal from each group was washed with saline and preserved in 10% buffered formaldehyde solution for histopathological studies. Paraffin-embedded tissue sections cut at 5 μm thickness were prepared and stained after deparaffination using hematoxylin and eosin stain (H and E). The various changes in histological features were graded as; Grade 0 (not present or very slight); Grade 1 (mild); Grade 2 (moderate) and Grade 3 (severe) as described earlier[1]. Photomicrographs were captured at a magnification of 40x and 100x.

Statistical analysis: All the results were expressed as Mean±SEM. Data analysis was performed using GraphPad Prism 5.0 software (GraphPad, San Diego, CA). Statistical comparisons were made between drug-treated groups and acetic acid control animals. Data analyzed using one-way ANOVA followed by Tukey's multiple range test. Data of macroscopical score and stool consistency was analyzed using nonparametric Kruskal-Wallis ANOVA. A value of $p<0.05$ was considered to be statistically significant.

RESULTS

Effect of ferulic acid on acetic acid-induced alterations on different parameters: Intrarectal instillation of 4% acetic acid resulted in significant decrease in body weight (187.7±3.47 g, $p<0.05$) and significant increase ($p<0.05$) in colon weight to length ratio (0.21±0.008), ulcer area (39.32±1.84 mm^2), ulcer index, macroscopic score (7.20±0.37) as well as stool consistency score (3.80±0.20) in acetic acid control rats as compared to normal rats. Ferulic acid (20 and 40 mg kg^{-1}) treatment for 12 days significantly ($p<0.05$) attenuated these acetic acid-induced alterations in body weight (205.3±5.43 and 213.6±6.65 g), colon weight to length ratio, ulcer area (18.42±1.26 and 6.54±1.32 mm^2), ulcer index, macroscopic score and stool consistency score as compared to acetic acid control rats. When compared with acetic acid control rats, treatment with prednisolone (2 mg kg^{-1}) showed a significant increase ($p<0.05$) in body weight (216.7±6.10 g) and significant decrease in the colon weight to length ratio (0.13±0.004), ulcer area (5.10±0.77 mm^2), ulcer index, macroscopic score as well as stool consistency score on the 12th day (Table 1).

Effect of ferulic acid on acetic acid-induced alterations in serum LDH and ALP: There was a significant increase ($p<0.05$) in serum LDH (2369.2±81.27 U l^{-1}) and ALP (157.2±6.08 U l^{-1}) of acetic acid control rats after intrarectal instillation of 4% acetic acid as compared to normal rats (675.4±39.99 and 65.08±3.36 U l^{-1}). When compared to acetic acid control rats, treatment with ferulic acid (20 and 40 mg kg^{-1}) significantly

Table 1: Effect of ferulic acid on acetic acid-induced alterations in body weight, colon weight to length ratio, ulcer area, ulcer index, macroscopic score and stool consistency score of rats

Treatment	Body weight (g)	Colon weight to length ratio	Ulcer area (mm^2)	Ulcer index	Inhibition (%)	Macroscopic score	Stool consistency score
Normal	223.4±5.31	0.11±0.005	0.00±0.00	0.00±0.00	-	0.00±0.00	0.40±0.24
Acetic acid control	187.7±3.47$^{\#}$	0.21±0.008$^{\#}$	39.32±1.84$^{\#}$	63.75±5.62$^{\#}$	-	7.20±0.37$^{\#}$	3.80±0.20$^{\#}$
Prednisolone (2)	216.7±6.10$^{*,\$}$	0.13±0.004$^{*,\$}$	5.10±0.77$^{*,\$}$	7.70±1.07$^{*,\$}$	87.91	2.80±0.37$^{*,\$}$	1.20±0.37$^{*,\$}$
Ferulic acid (10)	192.7±2.18	0.19±0.009	35.06±1.26	56.23±4.27	11.79	6.60±0.50	3.60±0.24
Ferulic acid (20)	205.3±5.43*	0.17±0.009*	18.42±1.26$^{*,\$}$	25.85±2.56$^{*,\$}$	48.62	5.20±0.37$^{*,\$}$	2.80±0.37$^{*,\$}$
Ferulic acid (40)	213.6±6.65$^{*,\$}$	0.12±0.004$^{*,\$}$	6.54±1.32$^{*,\$}$	9.36±1.91$^{*,\$}$	86.04	3.20±0.20$^{*,\$}$	1.60±0.40$^{*,\$}$

Data are expressed as Mean±SEM (n = 5) and analyzed by one way ANOVA followed by Tukey's multiple range test. Data of macroscopical score and stool consistency was analyzed using nonparametric Kruskal-Wallis ANOVA followed by Tukey's multiple range test. *$p<0.05$ as compared to acetic acid control group, $^{\#}p<0.05$ as compared to normal group as well as sham control group and $^{\$}p<0.05$ as compared to one another

Table 2: Effect of ferulic acid on acetic acid-induced alterations in serum LDH, serum ALP, colonic SOD, MDA and MPO of rats

Treatments	Serum LDH levels (U l^{-1})	Serum ALP levels (U l^{-1})	Colonic SOD (U mg^{-1} of protein)	Colonic GSH (µg mg^{-1} protein)	Colonic MDA (nM mg^{-1} of protein)	Colonic MPO (U mg^{-1})
Normal	675.4±39.99	65.08±3.36	14.23±2.28	28.18±1.35	24.72±1.67	4.92±0.79
Acetic acid control	2369.2±81.27$^{\#}$	157.20±6.08$^{\#}$	4.78±1.02$^{\#}$	15.48±0.87$^{\#}$	80.76±2.59$^{\#}$	25.04±1.10$^{\#}$
Prednisolone (2)	1211.0±92.62$^{*,\$}$	82.44±3.22$^{*,\$}$	12.14±2.88$^{*,\$}$	27.94±1.21$^{*,\$}$	33.22±2.01$^{*,\$}$	8.06±0.53$^{*,\$}$
Ferulic acid (10)	2135.4±87.06	145.30±8.48	5.96±0.89	17.56±1.08	73.66±2.80	23.32±0.92
Ferulic acid (20)	1579.2±60.04$^{*,\$}$	120.50±5.51$^{*,\$}$	7.89±1.12*	20.94±1.02*	57.62±2.70$^{*,\$}$	15.54±1.11$^{*,\$}$
Ferulic acid (40)	994.7±46.29$^{*,\$}$	78.20±5.45$^{*,\$}$	10.11±2.89$^{*,\$}$	24.44±1.02$^{*,\$}$	37.30±2.45$^{*,\$}$	10.02±0.53$^{*,\$}$

Data are expressed as Mean±SEM (n = 5) and analyzed by one way ANOVA followed by Tukey's multiple range test. *p<0.05 as compared to acetic acid control group, $^{\#}$p<0.05 as compared to normal group as well as sham control group and $^{\$}$p<0.05 as compared to one another

decreased (p<0.05) serum LDH (1579.2±60.04 and 994.7±46.29 U l^{-1}) and ALP (120.5±5.51 and 78.20±5.45 U l^{-1}) after 12 days of treatment. Prednisolone (2 mg kg^{-1}) treatment for 3 days also showed significant decrease (p<0.05) in the serum LDH (1211.0±92.62 U l^{-1}) and ALP (82.44±3.22 U l^{-1}) as compared to acetic acid control rats. But this decrease in serum LDH and ALP consistency was more significant (p<0.05) in ferulic acid (40 mg kg^{-1}) treated rats than prednisolone (2 mg kg^{-1}) treated rats (Table 2).

Effect of ferulic acid on acetic acid-induced alterations in colonic SOD, GSH, MDA and MPO: The levels of colonic SOD (4.78±1.02 U mg^{-1} of protein) and GSH (15.48±0.87 µg mg^{-1} protein) were significantly decreased (p<0.05) and colonic MDA (80.76±2.59 nM mg^{-1} of protein) and MPO (25.04±1.10 U mg^{-1}) were increased significantly (p<0.05) after intrarectal instillation of 4% acetic acid in the acetic acid control rats as compared to normal rats. Treatment with ferulic acid (20 and 40 mg kg^{-1}) for 12 days significantly increased (p<0.05) the levels of colonic SOD (7.89±1.12 and 10.11±2.89 U mg^{-1} of protein) and GSH (20.94±1.02 and 24.44±1.02 µg mg^{-1} protein), whereas, it significantly decreased (p<0.05) colonic MDA (57.62±2.70 and 37.30±2.45 nM mg^{-1} of protein) and MPO levels (15.54±1.11 and 10.02±0.53 U mg^{-1}) as compared to acetic acid control rats. When compared to acetic acid control rats, treatment with prednisolone (2 mg kg^{-1}) for 3 days also showed significant inhibition (p<0.05) in these alterations in colonic SOD (12.14±2.88 U mg^{-1} of protein), GSH (27.94±1.21 µg mg^{-1} protein), MDA (33.22±2.01 nM mg^{-1} of protein) and MPO (8.06±0.53U mg^{-1}). However, the inhibition of alteration in levels of colonic SOD, GSH, MDA and MPO were more significant (p<0.05) in prednisolone (2 mg kg^{-1}) treated rats as compared to ferulic acid-treated rats (Table 2).

Effect of ferulic acid on acetic acid-induced alterations in colonic HO-1 mRNA expression: Colonic HO-1 mRNA expression was significantly down-regulated (p<0.05) in acetic acid control rats as compared to normal rats. This down-regulation in colonic HO-1 mRNA expression was significantly inhibited (p<0.05) by ferulic acid (20 and 40 mg kg^{-1}) treatment as compared to acetic acid control rats. Prednisolone (2 mg kg^{-1}) treated rats also showed significant up-regulation (p<0.05) in the colonic HO-1 mRNA expression after 3 days of treatment as compared to acetic acid control rats. However, this up-regulation of colonic HO-1 mRNA expression was more significant (p<0.05) in the ferulic acid (40 mg kg^{-1}) treatment than prednisolone (2 mg kg^{-1}) treatment (Fig. 1).

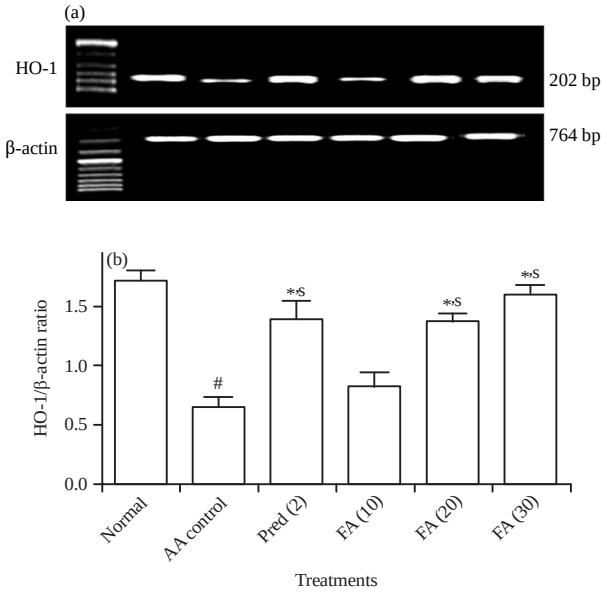

Fig. 1(a-b): Effect of ferulic acid on acetic acid-induced alterations in colonic HO-1 mRNA expression (A), quantitative representation of colonic HO-1 mRNA expression. Data are expressed as Mean±SEM (n = 5) and analyzed by one way ANOVA followed by Tukey's multiple range test. *p<0.05 as compared to acetic acid control group, $^{\#}$p<0.05 as compared to normal group as well as sham control group and $^{\$}$p<0.05 as compared to one another

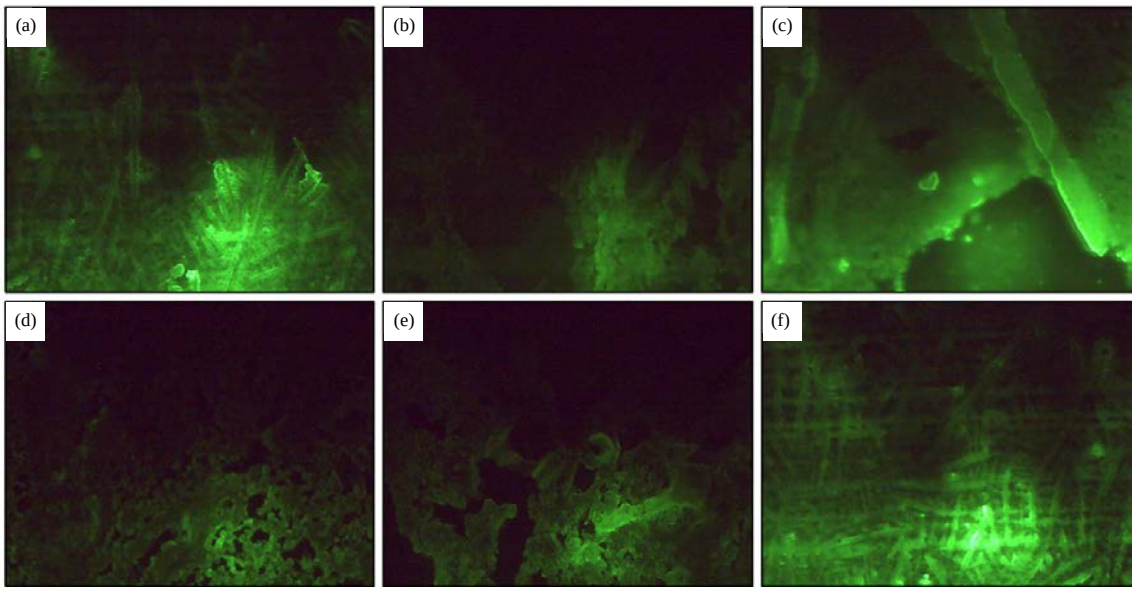

Fig. 2(a-f): Effect of ferulic acid on acetic acid-induced alterations in Nrf2 level. Photomicrographs of colon sections image of (a) Normal rat, (b) Acetic acid control rat, (c) Prednisolone (2 mg kg^{-1}) treated rat, (d) Ferulic acid (10 mg kg^{-1}) treated rat, (e) Ferulic acid (20 mg kg^{-1}) treated rat and (f) Ferulic acid (40 mg kg^{-1}) treated rat

Table 3: Effect of ferulic acid on acetic acid-induced pathological alterations in rat colon

			Lamina propria		Submucosa		
Treatments (dose in mg kg^{-1})	Mucosal epithelium Ulceration	Crypts Mucus depletion	Mononuclear infiltrate	Granulocyte infiltrate	Mononuclear infiltrate	Granulocyte infiltrate	Oedema
Normal	0	0	+	0	0	+	0
Acetic acid control	+++	+++	+++	+++	++	+++	+++
Prednisolone (2)	+	+	+	+	0	+	0
Ferulic acid (10)	+++	++	+++	+++	++	++	+++
Ferulic acid (20)	++	+++	++	+++	+	++	++
Ferulic acid (40)	+	0	+	0	+	+	+

0: No abnormality detected, +: Damage/active changes up to less than 25%, ++: Damage/active changes up to less than 50%, +++: Damage/ active changes up to less than 75% and ++++: Damage/active changes up to more than 75%

Effect of ferulic acid on acetic acid-induced alterations in Nrf2 level: Figure 2a qualitatively depicted presence of high level of Nrf2 in colon tissue from normal rats. Intrarectal instillation of 4% acetic acid resulted in a decrease in the level of Nrf2 in acetic acid control rats (Fig. 2b). Prednisolone (2 mg kg^{-1}) treated rats also showed the increased in the Nrf2 level as compared to acetic acid control rats (Fig. 2c). When compared to acetic acid control rats, 12 days treatment with ferulic acid (10, 20 and 40 mg kg^{-1}) showed increase in Nrf2 level (Fig. 2d-f).

Effect of ferulic acid on acetic acid-induced alterations in histology of colon: Colon tissue from normal rat showed intact epithelial crypts of mucosal layer without any evidence of infiltration of inflammatory cells (Fig. 3a). The histopathological features of acetic acid control rat included transmural necrosis (grade 3), edema (grade 3) and diffused inflammatory cell infiltration (grade 3) in the mucosa, desquamated areas and loss of the epithelium (Fig. 3b). Administration of prednisolone (2 mg kg^{-1}) (Fig. 3c) and ferulic acid (20 and 40 mg kg^{-1}) (Fig. 3e, f) attenuated the extent and severity of the histological signs of cell damage that were associated with intrarectal instillation of acetic acid. Colon tissue from ferulic acid (10 mg kg^{-1}) treated rats shows presence of inflammatory cells (grade 3) in lamina propria along with necrosis in epithelium (grade 3) (Fig. 3d and Table 3).

DISCUSSION

Acetic acid-induced experimental colitis model is widely used an animal model to determine the potential

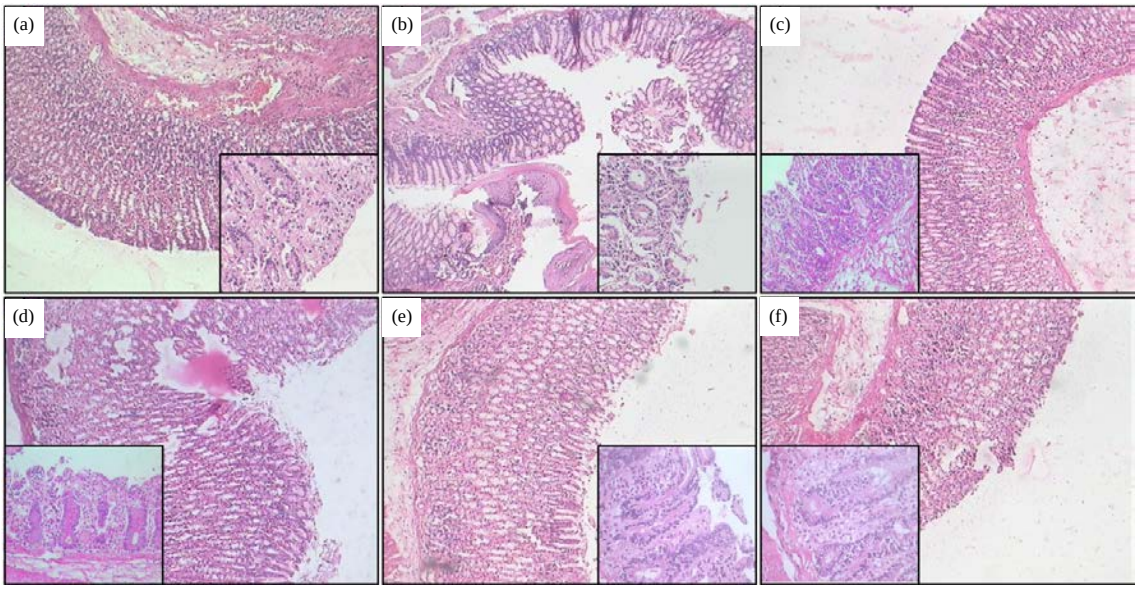

Fig. 3(a-f): Effect of ferulic acid on acetic acid-induced alterations in histology of colon. Photomicrographs of colon sections image of (a) Normal rat, (b) Acetic acid control rat, (c) Prednisolone (2 mg kg^{-1}) treated rat, (d) Ferulic acid (10 mg kg^{-1}) treated rat, (e) Ferulic acid (20 mg kg^{-1}) treated rat and (f) Ferulic acid (40 mg kg^{-1}) treated rat. H and E stain images (at 40x magnifications) and respective inset (at 100x magnifications) are typical and representative of each study group

of new therapeutic moieties against IBD[1]. It has been well-documented that levels of reactive oxygen species such as superoxide anion, hydrogen peroxide, hypochlorous acid and hydroxyl radical and nitrogen species are elevated in IBD patients thus oxidative stress plays an important role in initiation and maintenance of IBD[9,10]. In the present study, the anti-inflammatory potential of ferulic acid was investigated against colonic damage induced by acetic acid in rats by assessing various macroscopical, biochemical, histological and molecular changes.

Intrarectal administration of acetic acid cause release of acetate ions, which results in massive intracellular acidification, which in turn damage epithelial cells along with the rise in inflammatory response[1,14]. The vital role of neutrophil infiltration at the site of colonic damage in acetic acid-induced colitis has been well established and this elevated neutrophil responsible for the elevation of pro-inflammatory cytokine release[15]. The wet weight of the inflamed colon tissue is the hallmark of the severity and extent of inflammatory response, whereas, ulcer area and ulcer index depicted the healing potential of the drug. In the present investigation, intrarectal administration of acetic acid caused significant induction of ulcer in colon, which increases colon weight. However, administration of ferulic acid significantly inhibited acetic acid-induced ulcers as well as colon damage score.

It has been well documented that neutrophils are a central dogma for the production of superoxide anion and a cascade of various reactive species, which culminated in a vicious cycle of oxidative injury terminated into tissue necrosis and mucosal dysfunction[39-43]. Research carried out in clinical as well as preclinical setting proved that oxidative stress is an important mediator of the pathogenesis of ulcerative colitis[44]. Protonation of acetic acid in an intracellular microenvironment of the colonic epithelial cells causes conversion of O_2 is to H_2O_2 via SOD, which is an endogenous enzyme, thereafter it is converted to H_2O via catalase[15]. The GSH is a non-enzymatic antioxidant has potent electron donating capacity[45]. It plays an important role in controlling the redox state of the cell through several mechanisms, including scavenging of ROS and keeping the enzyme GSH peroxidase in a reduced state[46-48]. Acetic acid causes a reduction in colonic SOD and GSH levels, whereas ferulic acid demonstrated elevation of SOD and GSH levels providing credence to its antioxidant potential.

The MDA is a secondary end product of lipid peroxidation process, which results from polyunsaturated fatty acid peroxidation[49,50]. The MDA possess the ability to induce genotoxicity metabolic aberrations[51-53]. The MDA also causes DNA damage via formation of adducts and cross-links with DNA proteins[54-56]. In the present investigation, intrarectal administration of acetic acid caused an elevation in MDA

levels and this result is in line with the finding of previous investigators. Treatment with ferulic acid significantly inhibited acetic acid induced elevated MDA levels.

Myeloperoxidase (MPO) is an enzymatic catalyst stored in azurophilic granules of polymorphonuclear neutrophils and macrophages. The release of proinflammatory mediators caused activation of neutrophils, which elevates the MPO level[57-59]. The MPO synthesized and transported to lysosomes under stressful conditions from neutrophil leading to elevated oxidative stress[60-62]. Hence, MPO serves as a hallmark for neutrophil infiltration during stressful condition. It also has potential to impair vasodilatory and anti-inflammatory potential of NO via inhibiting endothelial NO concentration[63]. In this experiments, the colonic MPO activity was increased in acetic acid-treated animals. This notion was further supported by histological signs of inflammatory infiltration, edema and tissue injury. Ferulic acid showed a significant reduction in the level of MPO via its anti-inflammatory property.

In energy metabolism process, lactate dehydrogenase enzyme plays a pivotal role and its elevated levels in cell cytoplasm reflects cellular damage[1,64]. Whereas, elevated activity of alkaline phosphatase, a phosphohydrolase enzyme suggests inflammation during ulcerative colitis. The elevated level of these both enzymes i.e., LDH and ALP after intrarectal administration of acetic acid was significantly attenuated by ferulic acid administration, which might be due to its anti-inflammatory potential.

In heme degradation process, heme oxygenase-1 (HO-1) serves as rate-limiting enzyme. The HO-1 possesses the protective potential against the cytotoxicity induced by elevated oxidative stress and apoptotic cell death as well as inflammatory condition[65]. Furthermore, nuclear factor erythroid 2-related factor 2 (Nrf2), a bZIP transcription factor, is sequestered in the cytoplasm by Kelch-like ECH-associated protein 1 (Keap1). Translocation of Nrf2 from Keap1 to the nucleus occurs in presence of Antioxidant Response Element (ARE), which increases level of intracellular reactive oxygen species. In stressful condition, such as oxidative stress and inflammation, there is marked elevation in Keap1 level, which in turn impaired Nrf2 activity[66]. In present study mRNA expression of HO-1, as well as Nrf2 levels were significantly down-regulated in acetic acid control rats, whereas, ferulic acid treatment significantly increased these levels. Results of the present study are in accordance with the findings of the previous researcher, where ferulic acid showed an increased level of heme oxygenase-1[67].

CONCLUSION

The results demonstrate that ferulic acid exerts its healing potential on inflamed tissue in acetic acid induced colitis by virtue of its anti-inflammatory and anti-oxidant potential via upregulation in the HO-1 and Nrf-2 expressions.

ACKNOWLEDGEMENTS

The authors acknowledge Dr. S. S. Kadam, Vice-chancellor, Bharati Vidyapeeth University and Dr. K. R. Mahadik, Principal, Poona College of Pharmacy for the keen interest and providing the necessary facilities to carry out the study. The authors also acknowledge Invaluesys Research Group to carry out statistical analysis of the study.

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