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Protective effect of embelin against acetic acid induced ulcerative colitis in rats

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ABSTRACT

The aim of the present study is to evaluate the effect of embelin isolated from *Embelia ribes* on acetic acid induced colitis in rats. Experimental animals received embelin (25 and 50 mg/kg, p.o.) and sulfasalazine (100 mg/kg, p.o.) for five consecutive days before induction of colitis by intra-rectal acetic acid (3% v/v) administration and the treatment continued up to 7 days. The colonic mucosal injury was assessed by clinical, macroscopic, biochemical and histopathological examinations. Embelin treatment significantly decreased clinical activity score, gross lesion score, percent affected area and wet colon weight when compared to acetic acid induced controls. The treatment also reduced significantly the colonic myeloperoxidase activity, lipid peroxides and serum lactate dehydrogenase and significantly increased the reduced glutathione. The histopathological studies also confirmed the foregoing findings. The protective effect may be due to its antioxidant and anti-inflammatory activities.

1. Introduction

Inflammatory bowel disease is a group of multifactorial intestinal disorders of unknown etiology including Crohn's disease and ulcerative colitis. Both Crohn's disease and ulcerative colitis are common in Western populations and in urban rather than rural areas. Patients with these diseases are 10–20 times more likely to get cancer of the colon or bowel cancer (Nugent et al., 1991). The therapeutic strategy for treating inflammatory bowel disease now focuses on the use of anti-inflammatory agents (Xu et al., 2004). Glucocorticoids and aminosalicylate have been used for the treatment of inflammatory bowel disease, but their side effects remain a major clinical problem. Plant remedies play an important role in the therapy of many inflammatory disease conditions including inflammatory bowel disease (Mahgoub, 2003; Medhi et al., 2008).

A growing body of literature suggests that inflammatory bowel disease results from a dysregulated immune response to normal bacterial antigens. This uncontrolled immune system activation results in the sustained overproduction of reactive metabolites of oxygen and nitrogen. Some of the intestinal and/or colonic injury and dysfunction observed in inflammatory bowel disease is due to elaboration of these reactive species. In many studies, it has been reported that antioxidants showed beneficial effects on experimental colitis (Nosalova et al., 2000). Quinone derivatives from plants are known to possess protective effects against colitis induced by acetic acid (Mahgoub, 2003).

Embelin (2,5-dihydroxy-3-undecyl-1,4-benzoquinone), a major constituent of *Embelia ribes* Burm. (Family: Myrsinaceae) is a naturally occurring alkyl substituted hydroxy benzoquinone. The plant is indicated in traditional medicine for the treatment of various diseases (Varier, 2006). The fruit is bitter in taste, used to treat fever, inflammatory diseases and a variety of gastrointestinal ailments for thousands of years (Gupta et al., 1977). Embelin is reported to possess anti-inflammatory, analgesic (Chitra et al., 1994), antioxidant (Joshi et al., 2007) and wound healing (Kumara-Swamy et al., 2007) activities. It is also reported to impair the inflammatory signaling though inhibition of nuclear factor kappaB (NF-kappaB) activity (Ahn et al., 2007).

However, so far embelin was not tested for its protective action against inflammatory bowel disease though it is reported to possess potent antioxidant (Joshi et al., 2007) and anti-inflammatory (Chitra et al., 1994) activities. Various animal models of experimental colitis to screen drugs effective against inflammatory bowel disease have been established and acetic acid-induced colitis is an animal model that mimics some of the acute inflammatory responses seen in ulcerative colitis (Gonzalez et al., 1999). Hence, the present study aimed to assess the protective effect of embelin in the rat model of colitis induced by acetic acid.

2. Materials and methods

2.1. Extraction and isolation of embelin

The berries of *E. ribes* were purchased from Abirami Botanicals, Tuticorin, TN, India and authenticated by Medicinal Plants Survey and Collection Unit, Ootacamund, TN, India. Coarsely powdered berries (1 kg)

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were extracted for 72 h with n-hexane by cold maceration (3×2 l). The extract was concentrated to dryness in a rotavapor and chromatographed over silica gel (100–200 mesh). Elution with benzene and re-crystallization with ether (Chitra et al., 2003) afforded orange plates of embelin (yield 6.5 g, 0.325%). The percentage purity of embelin was checked by HPTLC when separated using the solvent system ethyl acetate:benzene (70:30) and characterized by comparing its physical and spectral values with those of literature (Feresin et al., 2003). The structure of embelin is depicted in Fig. 1.

2.2. Chemicals

Sulfasalazine (Panacea Biotec Ltd, New Delhi, India), 5,5-dithiobis-2-nitrobenzoic acid (Sigma Aldrich, St. Louis, USA), hexadecyltrimethylammonoium bromide, O-dianisidine hydrochloride, thiobarbituric acid (Hi-Media Laboratories Pvt. Ltd, Mumbai, India) and lactate dehydrogenase (ERBA Diagnostics, Mannheim, Germany) were purchased. All other chemicals used were of analytical grade.

2.3. Animals

Male Wistar rats (180–200 g) were obtained from the animal house of Sree Siddaganga College of Pharmacy, Tumkur, India, maintained under standard conditions (12 h light/dark cycle; 25±3 °C, 45–65% humidity) and had free access to standard rat feed and water *ad libitum*. All the animals were acclimatized to laboratory conditions for a week before commencement of the experiment. The experiments were performed during the light portion between 08:00 and 12:00 a.m. to avoid circadian influences. Animal studies were performed according to the prescribed guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India, India.

2.4. Preparation of suspensions

Different doses of embelin and the standard sulfasalazine were prepared as suspensions in distilled water using sodium carboxy methyl cellulose (sodium CMC, 0.3% w/v). Two doses of embelin (25 and 50 mg/kg) were selected for administration in oral route based on the earlier study (Chitra et al., 1994).

2.5. Induction of colitis

Animals were divided into six groups of six animals each. Group I served as normal control and received the vehicle (sodium CMC, 0.3% w/v). Group II received embelin alone (50 mg/kg b.w.). Group III served as colitis control and received only the vehicle (sodium CMC, 0.3% w/v). Group IV and V received embelin at 25 and 50 mg/kg b.w. Group VI was treated with standard sulfasalazine (100 mg/kg, p.o). All these treatments were given for seven days orally by using oral gavage. On the 4th day of the treatment, the animals were fasted overnight with access to water *ad libitum*. On the 5th day after 1 h of the aforementioned treatments, the animals (Groups III, IV, V and VI) were anesthetized by ether inhalation and a polypropylene tube with 2 mm diameter was inserted through the rectum into the colon to a distance of 8 cm. A solution of 2 ml of acetic acid (3%, v/v) in 0.9% saline was instilled into the lumen of the colon and maintained in a supine Trendelenburg position for 30 s to prevent the leakage of the intracolonic instillate. After 72 h of single dose administration of acetic acid (8th day), clinical activity scores were measured and the animals were anaesthetized with ether and blood was collected by retro orbital puncture for biochemical estimation. The animals were sacrificed by cervical dislocation and colon was dissected out. Colon was flushed gently with saline and weighed. It is used for macroscopic scoring, histopathological and biochemical estimations.

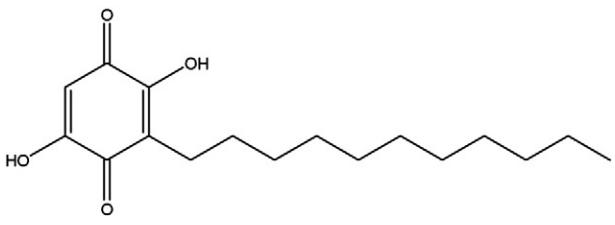

Fig. 1. Structure of embelin.

2.6. Evaluation of the disease

2.6.1. Clinical activity score

Colitis was quantified with a clinical score assessing weight loss, stool consistency and bleeding of the colon (measured by guaiac reaction, hemoccult) as described previously (Cooper et al., 1993). No weight loss was counted as 0 point, weight loss of 1 to 5% as 1 point, 5 to 10% as 2 points, 10 to 20% as 3 points, and 20% as 4 points. For stool consistency, the stool were collected in a thick paper and observed, 0 points were given for well formed pellets, 2 points for pasty and semiformed stools that did not stick to the anus, and 4 points for liquid stools that did stick to the anus. Bleeding was scored 0 points for no blood in hemoccult, 2 points for positive hemoccult, and 4 points for gross bleeding. These scores were added and divided by 3, forming a total clinical score that ranged from 0.0 (healthy) to 4.0 (maximal activity of colitis).

2.6.2. Macroscopic characters

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 faecal residues and weighed. Macroscopic inflammation scores were assigned based on clinical features of the colon using the following scoring pattern. No visible change was counted as 0 point, hyperemia at sites as 1 point, lesions having diameter l mm or less counted as 2 points, lesions having diameter 2 mm or less (number <5, 5–10 and >10) as 3, 4 and 5 points, respectively and lesions having diameter more than 2 mm (number <5, 5–10, >10) counted as 6, 7 and 8 points, respectively (Jagtap et al., 2004).

2.6.2.1. Scoring for rat caecum and colon. Rat caecum and colon (5 cm long) were scored for macroscopic features using the following scoring pattern. Score for an individual rat is calculated as the combined score of the colon and caecum. No percent area affected was counted as 0 point, 1–5% as 1 point, 5–10 as 2 points, 10–25 as 3 points, 25–50 as 4 points, 50–75 as 5 points and 75–100 was counted as 6 points, respectively (Jagtap et al., 2004).

2.7. Biochemical studies

A portion of colonic tissue (remaining from histopathological study) samples (n=6) were homogenized in 10% (w/v) of ice-cold potassium phosphate buffer (pH 7.4) using Elvenjan homogenizer (Remi Motors Ltd., Mumbai) and the homogenate was used for the measurement of myeloperoxidase activity (MPO), lipid peroxidation and reduced glutathione (GSH). In addition, serum lactate dehydrogenase (LDH) was also measured using the ERBA diagnostics kit.

2.7.1. Assessment of colonic MPO activity

The tissue homogenate was centrifuged at 800 ×*g* for 30 min at 4 °C. The supernatant was discarded. 10 ml of ice-cold 50 mM potassium phosphate buffer (pH 6.0), containing 0.5% hexadecyltrimethylammonium bromide and 10 mM EDTA was then added to the pellet. It was then subjected to one cycle of freezing, thawing and brief period (15 s) of sonication. After sonication, the solution was centrifuged at 13,100 ×*g* for 20 min. The MPO activity was measured spectrophotometrically (Krawisz et al., 1984). 0.1 ml of supernatant was combined with 2.9 ml of 50 mM

phosphate buffer containing 0.167 mg/ml of O-dianisidine hydrochloride and 0.0005% hydrogen peroxide. The change in absorbance was measured at 460 nm. One unit of MPO activity is defined as the change in absorbance per min by 1.0 at room temperature, in the final reaction. It has been calculated by using the following formula

$$\text{MPO activity (U/g)} = X/\text{weight of the piece of tissue taken}$$

Where X = 10 × change in absorbance per min/volume of supernatant taken in the final concentration.

2.7.2. Measurement of colonic lipid peroxides concentration

Thiobarbituric acid reactive substance in the homogenate was estimated by using standard protocol. Briefly, the homogenate was incubated with 15% trichloroacetic acid, 0.375% thiobarbituric acid and 5N HCL at 95 °C for 15 min, the mixture was cooled, centrifuged and the absorbance of the supernatant was measured at 532 nm against appropriate blank. The amount of lipid peroxides was determined by using $\varepsilon = 1.56 \times 10^5\ M^{-1}\ cm^{-1}$ and expressed as μmol/g of wet tissue weight (Ohkawa et al., 1979).

2.7.3. Determination of colonic GSH contents

The assay is based on the formation of a relatively stable yellow product when sulphydryl groups react with 5,5-dithio-bis-2-nitro-benzoic acid (DTNB). Briefly, proteins were precipitated using 10% TCA, centrifuged and 0.5 ml of the supernatant was mixed with 0.2 M phosphate buffer (pH 8.0) and 10 mM DTNB. This mixture was incubated for 10 min and the absorbance was measured at 412 nm against appropriate blank (Moran et al., 1979). The results were expressed as nmol/g of wet tissue weight.

2.8. Histolopathological study

A portion (2 cm) of the colonic specimen from each rat (n = 6) was fixed in 10% formalin, cut into 5 μm thickness, stained using heamatoxylin–eosin and histopathological observations were made. The stained sections of colon were examined for any inflammatory changes like infiltration of the cells, necrotic foci and damage to tissue structures like payers patches, damage to nucleus, etc.

2.9. Statistical analysis

The values were expressed as mean ± S.E.M. The statistical analysis was carried out by one way analysis of variance (ANOVA) followed by multiple comparison test of Tukey–Kramer. P values < 0.05 were considered as significant.

3. Results

3.1. Clinical and macroscopic activity score

Acetic acid caused severe macroscopic edematous inflammation in the colon. The clinical score, gross lesion score, percent area affected, wet colon weight for colitis control group were found to be 3.35 ± 0.39, 4.80 ± 0.58, 64.80 ± 12.91 and 142.80 ± 3.02, respectively (Table 1). Embelin at both the doses (25 and 50 mg/kg b.w.) showed significant decrease in the clinical activity scores (1.98 ± 0.12 and 1.20 ± 0.13, P < 0.001), gross lesion score (2.40 ± 0.68 and 1.80 ± 0.37, P < 0.01 and P < 0.001), percent affected area (16.40 ± 8.80 and 12.00 ± 5.39%, P < 0.001) and wet colon weight (117.60 ± 3.41 and 116.80 ± 8.38 mg/cm, P < 0.05 and P < 0.01), respectively. However, the standard sulfasalazine (100 mg/kg b.w.) showed better results in all these parameters, indicating its potent activity at the dose tested. Embelin treated alone at 50 mg/kg b.w. caused no significant change in these parameters when compared to normal control.

3.2. Biochemical studies

Intra-rectal administration of acetic acid showed a significant increase in the concentrations of MPO and lipid peroxides (4.27 ± 0.20 U/g and 14.48 ± 0.36 μmol/g of wet tissue, P < 0.001), decreased the GSH level (821.93 ± 15.89 nmol/g of wet tissue, P < 0.001) in colonic tissue and increased the LDH in serum (2002.00 ± 96.60 U/l, P < 0.001) when compared to normal control. The pre-treatment with embelin at 25 and 50 mg/kg b.w. significantly (P < 0.001) reduced the alterations in these biochemical parameters when compared to colitis control and towards the normal level (Fig. 2). Embelin at 50 mg/kg b.w. was found to be more potent and the activity was comparable to standard sulfasalazine at 100 mg/kg b.w. treatment. When treated embelin alone at 50 mg/kg b.w. caused no significant change in these biochemical parameters when compared to normal indicating its safety.

3.3. Histopathological studies

Acetic acid induced colitis showed massive necrotic destruction of epithelium, submucosal edema, areas of haemorrhages and inflammatory cellular infiltration. Embelin at low dose level showed minimal damage of the mucosa with slight submucosal edema and mild inflammatory cell infiltration. Embelin at 50 mg/kg b.w. and sufasalazine at 100 mg/kg b.w. showed remarkable recovery of colonic mucosa from acetic acid induced colitis damage (Fig. 3).

4. Discussion

Acetic acid induced colitis is a model wherein inflammatory mediators such as reactive oxygen species, vasoactive amines and eicosanoids play a prominent role (Carty et al., 2000). The underlying pathophysiological mechanisms involved include colon structure and mucosa barrier destruction by chemical stimulation, enhanced vessel permeability, increased inflammatory mediators, promotion of fibrin hydrolysis, and disturbance of cruor process.

The wet weight of the inflamed colon tissue is considered a reliable and sensitive indicator of the severity and extent of inflammatory response (Rachmilewitz et al., 1989). In the present study, pre-treatment with embelin in the acetic acid induced colitis significantly reduced the wet weight of colon, clinical activity, gross lesion score and percentage of affected area compared with colitis control, indicating its protective effect from ulcerative colitis.

Table 1
Clinical and macroscopic characters of embelin treated acetic acid induced colitis in rats.

Treatment	Clinical score (% protection)	Gross lesion score (% protection)	Area affected (%)	Wet colon weight/length (mg/cm)
Normal control	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	107.80 ± 4.03
Embelin (50 mg/kg, p.o.)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	95.00 ± 5.17
Colitis control (2 ml of 3% acetic acid, intra-rectal)	3.35 ± 0.39[a]	4.80 ± 0.58[a]	64.80 ± 12.91[a]	142.80 ± 3.0[b]
Acetic acid + embelin (25 mg/kg, p.o.)	1.98 ± 0.12[c] (40.90)	2.40 ± 0.68[d] (50.00)	16.40 ± 8.80[c]	117.60 ± 3.41[e]
Acetic acid + embelin (50 mg/kg, p.o.)	1.20 ± 0.13[c] (64.18)	1.80 ± 0.37[c] (62.50)	12.00 ± 5.39[c]	116.80 ± 8.38[d]
Acetic acid + sulfasalazine (100 mg/kg, p.o.)	0.89 ± 0.06[c] (73.43)	0.83 ± 0.16[c] (82.70)	4.00 ± 1.00[c]	112.00 ± 3.80[d]

Values are given as mean ± S.E.M. for groups of six animals each, Tukey–Kramer; values are statistically significant at [a]P < 0.001, [b]P < 0.01 between normal and colitis control, [c]P < 0.001, [d]P < 0.01, [e]P < 0.05 between colitis control and treated groups.

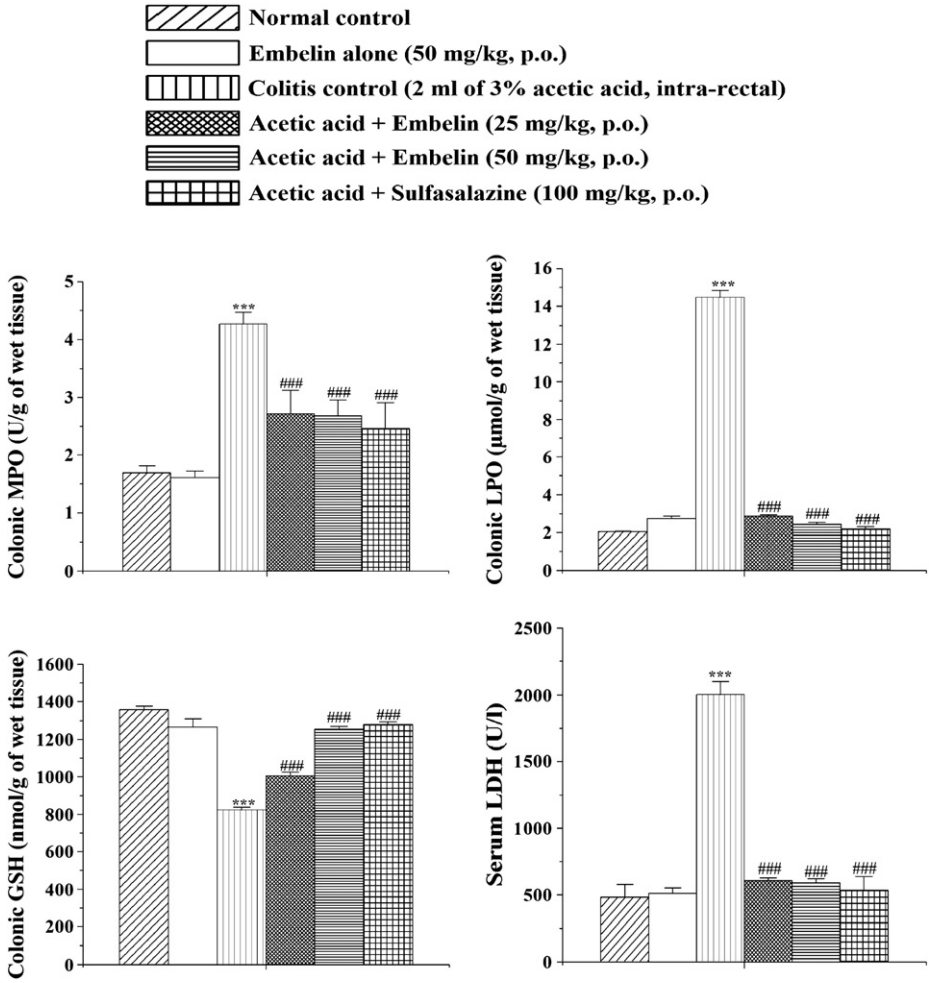

Fig. 2. Effect of embelin on biochemical profile in acetic acid induced colitis in rats. Values are given as mean ± S.E.M. for groups of six animals each, Tukey–Kramer; values are statistically significant at $^{***}P<0.001$ between normal and colitis control and $^{###}P<0.001$ between colitis control and treated groups.

Myeloperoxidase is an enzyme present in neutrophils and at a much lower concentration in monocytes and macrophages. The level of MPO activity is directly proportional to the neutrophil concentration in the inflamed tissue. Therefore, a measurement of MPO activity has been considered a quantitative and sensitive assay for acute intestinal inflammation. In addition, increased MPO activity has been reported to be an index of neutrophil infiltration and inflammation (Choudhary et al., 2001). Embelin at both the doses exhibited a significant decrease in the MPO levels when compared to acetic acid induced colitis control.

Increased lipid peroxides that occurs in colonic tissue can initiate a vicious cycle that generates more and more reactive metabolites, which exhausts cellular antioxidants, vitamin C and E and ultimately favors the consequent development of further inflammation and ulceration. Embelin at both the doses significantly inhibited the increase in the lipid peroxides activity in the colonic tissue. It is therefore reasonable to assume that the embelin treatment improves colonic oxidative balance in colitis induced animals, because embelin was able to reduce the level of malondialdehyde, a good indicator of lipid peroxidation (Ohkawa et al., 1979). GSH is involved in the synthesis and repair of DNA, assists the recycling of vitamins C and E, blocks free radical damage, enhances the antioxidant activity of vitamin C, facilitates the transport of amino acids and play a critical role in detoxification (Chavan et al., 2005). Pre-treatment of embelin reversed the depletion of GSH and restored the levels towards the normal. LDH, a cytosolic enzyme is involved in the biochemical regulation reaction of the body tissues and fluids. An elevation of LDH in serum indicates a shift towards anaerobiosis resulting in the enhanced production of lactic acid (Manna et al., 2004). In the present study, the embelin pre-treatment altered the serum LDH level induced by acetic acid towards the normal.

The clinical, macroscopic and biochemical evidence for the protective effect of embelin on acetic acid induced colitis in rats was well correlated by the histopathological studies. The histological science of inflammation such as leukocyte infiltration, edema and tissue injury was found to be low following the pre-treatment with embelin.

The results obtained from embelin treated acetic acid induced colitis in the present study are in well correlation with earlier results of its ability to inhibit carrageenan induced paw edema in rats, inhibition of NF-kappaB activation, which makes it a potentially effective suppressor of tumor cell survival, proliferation, invasion, angiogenesis and inflammation (Gupta et al., 1977; Chitra et al., 1994; Ahn et al., 2007). In addition, embelin is known to suppress the NF-kappaB activation induced by TNF-α and various other inflammatory and carcinogenic agents (Ahn et al., 2007). The results from the present study are also in consistent with the earlier study of its ability to scavenge physiologically relevant oxidizing radicals (Joshi et al., 2007).

In conclusion, the present data suggest that the pre-treatment of embelin prevents acetic acid induced colitis in rats and this protective effect may at least in part be due to its antioxidant and anti-inflammatory actions. However, further investigations are necessary to evaluate whether a similar efficacy can be achieved in other models of experimental colitis that simulate human inflammatory bowel disease and also evaluate the post-treatment effect of embelin in acetic acid induced colitis.

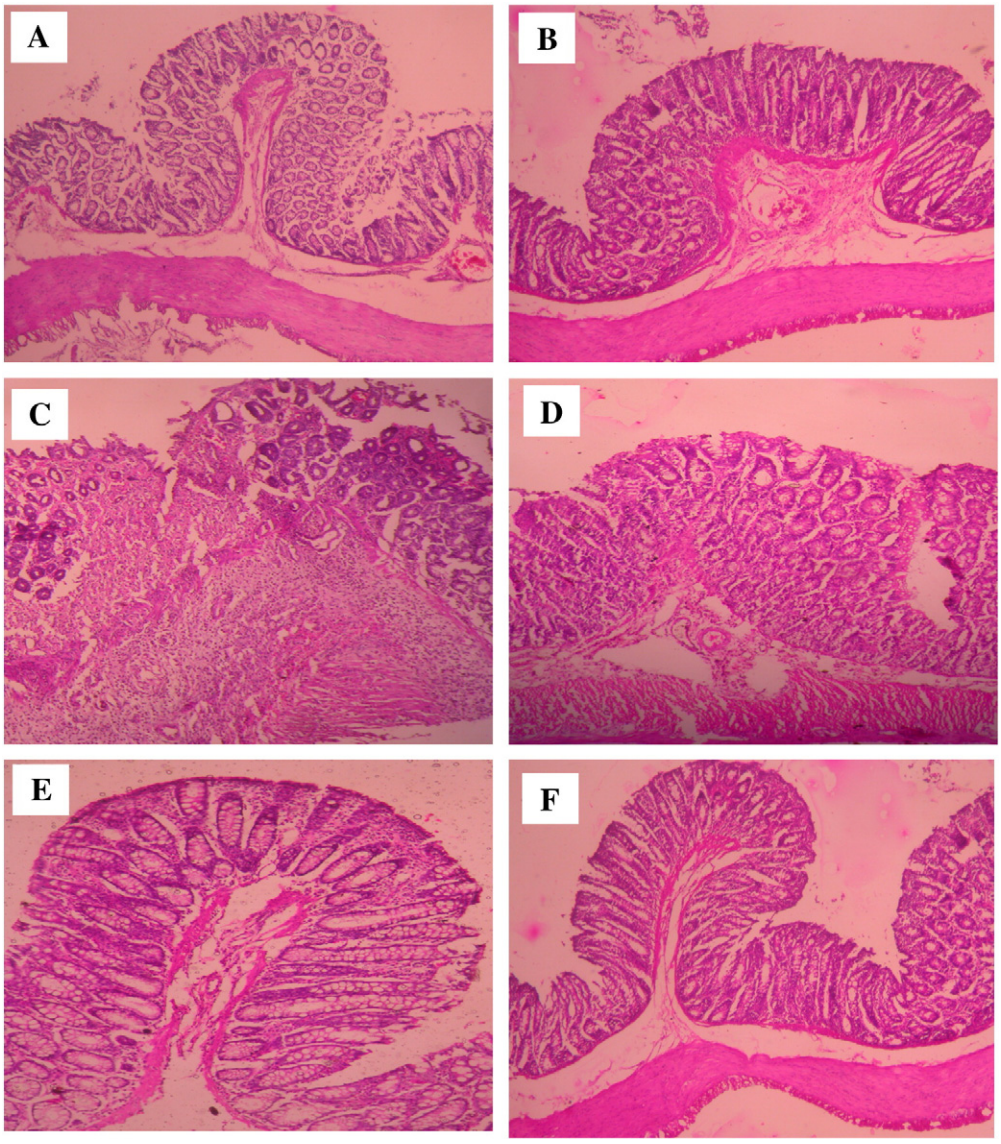

Fig. 3. Histopathological changes in colon of experimental rats. A and B: Normal intact mucosa from normal control and embelin alone treated animals showing intact epithelial surface. C: Acetic acid induced colitis showing massive necrotic destruction of epithelium, submucosal edema, areas of haemorrhages and inflammatory cellular infiltration. D: Acetic acid + embelin 25 mg/kg showing minimal damage of the mucosa with slight submucosal edema and mild inflammatory cell infiltration. E: Acetic acid + embelin 50 mg/kg showing significant protection of colonic mucosa from acetic acid induced colitis damage. F: Acetic acid + sufasalazine 100 mg/kg showing normal colonic structure.

Conflict of interest

There are no conflicts of interest.

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References

Ahn, K.S., Sethi, G., Aggarwal, B.B., 2007. Embelin, an inhibitor of X chromosome linked inhibitor of apoptosis protein, blocks nuclear factor-kB (NF-kB) signaling pathway leading to suppression of NF-kB regulated antiapoptotic and metastatic gene products. Mol. Pharmacol. 71, 209–219.

Carty, E., De-Brabander, M., Feakins, R.M., Rampton, D.S., 2000. Measurement of *in vivo* rectal mucosal cytokine and eicosanoid production in ulcerative colitis using filter paper. Gut 46, 487–492.

Chavan, S., Sava, L., Saxena, V., Pillai, S., Sontakke, A., Ingole, D., 2005. Reduced glutathione: importance of specimen collection. Indian J. Clin. Biochem. 20, 150–152.

Chitra, M., Shyamaladevi, C.S., Viswanathan, S., Sukumar, E., 2003. Effect of embelin on lipid profile in transplanted fibrosarcoma in rats. Indian J. Pharmacol. 35, 241–244.

Chitra, M., Sukumar, E., Suja, V., Devi, C.S.S., 1994. Antitumor, anti-inflammatory and analgesic property of embelin, a plant product. Chemotherapy 40, 109–113.

Choudhary, S., Keshavarzian, A., Yong, M., Wade, M., Bocckino, S., Day, B.J., Banan, A., 2001. Novel antioxidants zolimid and AEOL11201 ameliorate colitis in rats. Dig. Dis. Sci. 46, 2222–2230.

Cooper, H.S., Murthy, S.N., Shah, R.S., Sedergran, D.J., 1993. Clinicopathologic study of dextran sulfate sodium experimental murine colitis. Lab Invest. 69, 238–249.

Feresin, G.E., Tapia, A., Sortino, M., Zacchino, S., Arias, A.R., Inchausti, A., Yaluff, G., Rodriguez, J., Theoduloz, C., Schmeda Hirschmann, G., 2003. Bioactive alkyl phenols and embelin from *Oxalis erythrorhiza*. J. Ethnopharmacol. 88, 241–247.

Gonzalez, R., Rodriguez, S., Romay, C., Ancheta, O., Gonzalez, A., 1999. Anti-inflammatory activity of Phyocyanin extract in acetic acid-induced colitis in rats. Pharmacol. Res. 39, 55–59.

Gupta, O.P., Ali, M.M., Ray Ghatak, B.J., Atal, C.K., 1977. Some pharmacological investigations of embelin and its semisynthetic derivatives. Indian J. Physiol. Pharmacol. 21, 31–39.

Jagtap, A.G., Shirke, S.S., Phadke, A.S., 2004. Effect of polyherbal formulation on experimental models of inflammatory bowel diseases. J. Ethnopharmacol. 90, 195–204.

Joshi, R., Kamat, J.P., Mukherjee, T., 2007. Free radical scavenging reactions and antioxidant activity of embelin: biochemical and pulse radiolytic studies. Chem. Biol. Interact. 167, 125–134.
Krawisz, J.E., Sharon, P., Stenso, W.F., 1984. Qualitative assay for acute intestinal inflammation based on myeloperoxidase activity. Gastroenterology 87, 1344–1350.
Kumara-Swamy, H.M., Krishna, V., Shankarmurthy, K., Abdul-Rahiman, B., Mankani, K.L., Mahadevan, K.M., Harish, B.G., Raja Naika, H., 2007. Wound healing activity of embelin isolated from the ethanol extract of leaves of *Embelia ribes* Burm. J. Ethnopharmacol. 109, 529–534.
Mahgoub, A.A., 2003. Thymoquinone protects against experimental colitis in rats. Toxicol. Lett. 143, 133–143.
Manna, S., Bhattacharyya, D., Basak, D.K., Mandal, T.K., 2004. Single oral dose toxicity study of α-cypermethrin in rats. Indian J. Pharmacol. 36, 25–28.
Medhi, B., Prakash, A., Avti, P.K., Saikia, U.N., Pandhi, P., Khanduja, K.L., 2008. Effect of manuka honey and sulfasalazine combination to promote antioxidant defense system in experimentally induced colitis model in rats. Indian J. Exp. Biol. 46, 583–590.
Moran, A., Depierre, J.W., Mannervick, B., 1979. Levels of glutathione, glutathione reductase, glutathione-S-transferase activities in rat liver. Biochim. Biophys. Acta 582, 67–68.
Nosalova, V., Cerna, S., Bauer, V., 2000. Effect of N-acetylcysteine on colitis induced by acetic acid in rats. Gen. Pharmacol. 35, 77–81.
Nugent, F.W., Haggitt, R.C., Gilpin, P.A., 1991. Cancer surveillance in ulcerative colitis. Gastroenterology 100, 1241–1248.
Ohkawa, H., Ohishi, N., Yagi, K., 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 95, 351–358.
Rachmilewitz, D., Simon, P.L., Schwartz, L.W., Griswald, D.E., Fondacaro, J.D., Wasserman, M.A., 1989. Inflammatory mediators of experimental colitis in rats. Gastroenterology 97, 326–327.
Varier, P.S., 2006. Indian Medicinal Plants, a Compendium of 500 Species. Orient Longman (Pvt) Ltd, Chennai, India. 368–371.
Xu, C.T., Meng, S.Y., Pan, B.R., 2004. Drug therapy for ulcerative colitis. World J. Gastroenterol. 10, 2311–2317.