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Effect of *Emblica officinalis* Methanolic Fruit Extract on Indomethacin Induced Enterocolitis in Rats

[1]C.D. Deshmukh, [1]A.T. Pawar and [2]V. Bantal
[1]Department of Pharmacology, MAEER's Maharashtra Institute of Pharmacy, Kothrud, Pune-411038, Maharashtra, India
[2]Department of Pharmacology, Jawaharlal Nehru Medical College, KLE University, Belgaum 590010, Karnataka, India

Abstract: The present study investigated the protective effect of the methanolic extract of fruit of *Emblica officinalis* (MEO) against indomethacin induced enterocolitis in male Wistar rats. Enterocolitis was induced by subcutaneous administration of indomethacin solution (7.5 mg kg^{-1}, 2 days) for 2 days. The study comprised of 5 groups (n = 6), normal saline-treated, enterocolitis induced, MEO treated groups (100 and 200 mg kg^{-1}, p.o.) and sulfasalazine treated (500 mg kg^{-1}, p.o.) group. On the fourth day animals were sacrificed by cervical dislocation and dissected open to remove gastrointestinal tract. Ileum and colon pieces (10 cm long) are assessed for inflammation on macroscopic score and histopathological studies. Pretreatment with MEO has shown a decreased in macroscopic scores for inflammation as compared to indomethacin treated group. A significant decrease in serum lactate dehydrogenase level was also observed in MEO treated groups. Histopathology examination of MEO treated group revealed less damage compared to indomethacin treated group. The finding of present study provides evidence that *Emblica officinalis* may be beneficial in patients with inflammatory bowel diseases.

Key words: *Emblica officinalis*, indomethacin, enterocolitis, inflammatory bowel diseases, lactate dehydrogenase

INTRODUCTION

Inflammatory Bowel Diseases (IBD) including Ulcerative Colitis (UC) and Crohn's disease are amongst the most challenging human illness in the world. The etiology of IBD still remains incompletely understood, but it is generally agreed that a complex interplay between genetic, environmental and immunological factors contributes to the initiation and progression of the disease (Wirtz and Neurath, 2007).

As the exact etiology and pathogenesis of the disease development is unclear, the treatment of IBD becomes major obstacle. Commonly used medications are aminosalicylates, glucocorticoids, antibiotics and immunomodulators (Podolsky, 1991; Strober *et al*., 1998; Shanahan, 2001). Many drugs which are used in IBD offer temporary relief and/or maintain remission (Jagtap *et al*., 2004).

Corresponding Author: Anil T. Pawar, Department of Pharmacology, MAEER's Maharashtra Institute of Pharmacy, Kothrud, Pune-411038, Maharashtra, India
Tel: +91-020-30273653 Fax: +91-020-25460616

Current treatment is not much satisfactory to treat the disease so various herbal and natural plants like *Gingko biloba* (Harputluoglu *et al*., 2006), *Copaifera langsdorffii* (Paiva *et al*., 2004) polyherbal formulation containing *Aegle marmeloes*, *Coriandrum sativum*, *Cyperus rotundus* and *Vetiveria zinzanioids* (Jagtap *et al*., 2004) has proved to be effective in IBD due to their antioxidant and anti-inflammatory action. Thus, the use of medicinal plants or their active components has become an increasingly attractive approach for the treatment of enterocolitis.

Emblica officinalis Gaertn. (family: Euphorbiaceae) is an herbal plant widely used in many of the indigenous medical preparations against variety of the diseases. It is the major ingredient of Chyavanprash which is used as health tonic (Jose and Kuttan, 2000). The earlier study have demonstrated potent antimicrobial (Ahmad *et al*., 1998), anti-diabetic (Sabu and Kuttan, 2002), antitussive (Nosal'ova *et al*., 2003), adaptogenic (Rege *et al*., 1999), hepatoprotective (Jose and Kuttan, 2000), antioxidant (Bhattacharya *et al*., 1999; Bandyopadhyay *et al*., 2000; Bafna and Balaraman, 2005), antitumour (Jose and Kuttan, 2001), radioprotective (Jagetia *et al*., 2002), anti-ulcerogenic (Sairam *et al*., 2002), anti-pyretic and analgesic (Perianayagam *et al*., 2004) activities of *Emblica officinalis*. Leaf and fruit extracts have also been shown to possess potent anti-inflammatory activity (Asmawi *et al*., 1993; Vormisto *et al*., 1997; Sharma *et al*., 2003).

Keeping in view the potent anti-inflammatory activity of *Emblica officinalis* observed in experimental studies, this study was undertaken to investigate its anti-inflammatory effect in indomethacin induced enterocolitis in male Wistar rats.

MATERIALS AND METHODS

Work Duration

The study was conducted between June 2008 to January 2009 in the Department of Pharmacology, Jawaharlal Nehru Medical College, KLE University, Belgaum, India.

Animals

Male Wistar albino rats weighing between 200-250 g each were used for the experiment. They were procured from Indian Institutes of Sciences, Bangalore, India. They were placed singly in cages with wire net floor and maintained at 27±2°C, relative humidity 65±10% under 12 h light/dark cycles. The animals were given standard diet supplied by Pranav Agro Industries Ltd., Sangli, India. The study protocol was approved from the Institutional Animal Ethics Committee constituted in accordance with the rules and guidelines of the CPCSEA (Committee for the purpose of Control and Supervision of Experiments on Animals), India.

Plant Material and Extraction

Fresh fruits of *Emblica officinalis* were purchased from Bhopal, authenticated by Research officer at Regional Medical Research Centre, Belgaum (Voucher No. RMRC467). The fruits were washed, dried and crushed. The crushed material was extracted with 80% methanol by Soxhlet apparatus. The extract obtained was evaporated to dryness under low pressure and stored in refrigerator for pharmacological studies. The yield of the extract was 30% w/w.

Drugs

Indomethacin was obtained from, Indoco Pharmaceuticals, Mumbai, India. Sulfasalazine was supplied by Wallace Pharmaceuticals, Goa, India. Indomethacin and

sulfasalazine was supplied as a gift samples for research purpose. All other chemicals and reagents used were of analytical grade and procured from approved chemical suppliers.

Experimental Design

Thirty animals were randomly divided into five groups, containing six animals in each. Group 1 served as a normal control which did not received any treatment and maintained on regular rat food and drinking water *ad libitum*. All remaining groups received 7.5 mg kg^{-1} indomethacin (solubilized in 100% alcohol and then diluted with 5% w/v sodium bicarbonate solution) on two consecutive days subcutaneously. Group 2 was indomethacin treated animals without any treatment. Group 3 and 4 served as MEO treatment group, received MEO in a dose of 100 and 200 mg kg^{-1}, respectively for 7 days and indomethacin on 8th and 9th day. The MEO treatment was continued till 11th day. Group 5, standard drug treated group, which received indomethacin and treated with sulfasalazine (500 mg kg^{-1}, p.o., for 4 days). The MEO was dissolved in distilled water whereas sulfasalazine was suspended in distilled water using 0.5% w/v carboxy methyl cellulose solution and were administered by oral route.

After treatment period, animals were sacrificed by cervical dislocation and dissected to remove GIT (Duodenum to anus). The ileum and colon part were scored for inflammation based on microscopic features. Tissue specimens were kept in 10% formalin for macroscopic and histopathological studies. Blood samples were centrifuged; serum were separated and used for assay of lactate dehydrogenase (LDH).

Assessment of Enterocolitis

Macroscopic Scoring

Rat ileum and colon pieces (10 cm long each) were removed and cut longitudinally, slightly cleaned in physiological saline to remove faecal residues. Macroscopic inflammation scores were assigned based on clinical features of the colon using an arbitrary scale ranging from 0-5 as follows: (0) No visible change, (1) hyperemia at sites, (2) lesions having diameter 1 mm or less, (3) lesions having diameter 2 mm or less (No. <5), (4) lesions having diameter 2 mm or less (No. 5-10), (5) lesions having diameter 2 mm or less (No. >10) (Jagtap *et al*., 2004).

Histopathological Studies

To process for histopathological studies, colonic specimens were fixed in 10% formalin in phosphate buffered saline, embedded in paraffin and cut into 4 μm sections. Paraffin sections were deparaffinized with xylene, hydrated and stained with hematoxylin and eosin (H and E). The stained sections were assessed for any inflammatory changes including infiltration of cells, necrosis or damage to nucleus or tissue structures etc. (Jagtap *et al*., 2004).

Measurement of Serum LDH

Serum LDH was assayed according to the method previously described (King, 1965). Briefly, 0.1 mL enzyme preparation was added to 0.1 mL of buffer substrate and the tubes were incubated at 37°C for 15 min. After adding 0.2 mL NAD^+ solution, the incubation was continued for another 15 min. The reaction was arrested by adding 0.1 mL of DNPH (2, 4-dinitrophenyl hydrazine). The tubes were incubated for a further 15 min at 37°C and then 7 mL 0.4 M NaOH (sodium hydroxide) solution was added. The intensity of color developed was measured at 420 nm in a UV spectrophotometer (Shimadzu Scientific Instruments, UV-3600). The activity of enzyme was expressed as μmol pyruvate liberated (mg protein)$^{-1}h^{-1}$.

Statistical Analysis

The results were expressed as Mean±Standard Error Mean (SEM). The statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons test and $p<0.01$ was considered significant.

RESULTS AND DISCUSSION

Effect of MEO on Macroscopic Scores

Two days treatment with indomethacin (7.5 mg kg^{-1}, s.c.) produced severe inflammation in rat intestine. Jejunum and proximal ileum showed more inflammation compared to rest portion of small intestine and ileum. Ileocecal region was most severely affected showing severe ulcers, necrosis and hemorrhagic spots. The ileum showed many lesions with skip areas in between of normal tissue. The MEO and sulfasalazine treated groups showed lower score values compared to indomethacin treated group (Table 1).

Effect of MEO on Serum LDH Levels

Serum LDH levels was significantly elevated following indomethacin administration compared to normal animals (2525±62.03 vs. 1899±66.56 U L^{-1}, respectively, $p<0.001$). Treatment with MEO (both 100 and 200 mg kg^{-1}) and sulfasalazine reduced the elevated serum LDH level induced by indomethacin (2219±33.06, 2038 ±25.85, 2310±82.27 vs. 2525±62.03 U L^{-1}, respectively $p<0.001$) Fig. 1.

Table 1: Effect of *Emblica officinalis* on macroscopic scores of rat ileum and colon

Groups	Treatment	Macroscopic scores of ileum	Macroscopic scores of colon
Normal	Saline (0.5 mL kg^{-1})	0.00±0.00	0.00±0.00
Indomethacin treated	Indomethacin (7.5 mg kg^{-1})	7.17±0.31[a]	3.67±0.33[a]
Standard drug treated	Indomethacin + Sulfasalazine	4.00±0.37[b]	1.83±0.31[b]
MEO treated	Indomethacin + MEO (100 mg kg^{-1})	4.84±0.31[b]	2.16±0.31[c]
MEO treated	Indomethacin + MEO (200 mg kg^{-1})	3.84±0.31[b]	1.33±0.21[b]

Data were expressed as Mean±SEM, n = 6. [a]$p<0.001$ vs normal group. [b]$p<0.001$, [c]$p<0.01$ vs. indomethacin treated group

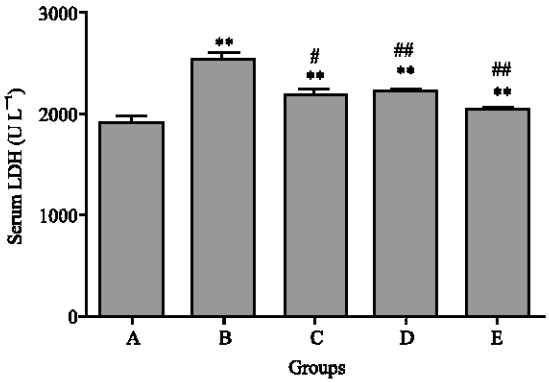

Fig. 1: Effect of MEO on serum Lactate Dehydrogenase (LDH) levels (U L^{-1}) in indomethacin induced enterocolitis in rats. A: Normal control group, B: Indomethacin treated group, C: Standard drug (Sulfasalazine: 500 mg kg^{-1}) treated group, D: MEO treated (100 mg kg^{-1}), E: MEO treated (200 mg kg^{-1}) group. Results are expressed as Mean±SEM, n = 6/group, **$p<0.001$, vs. normal group. #$p<0.01$, ##$p<0.001$ vs. indomethacin group

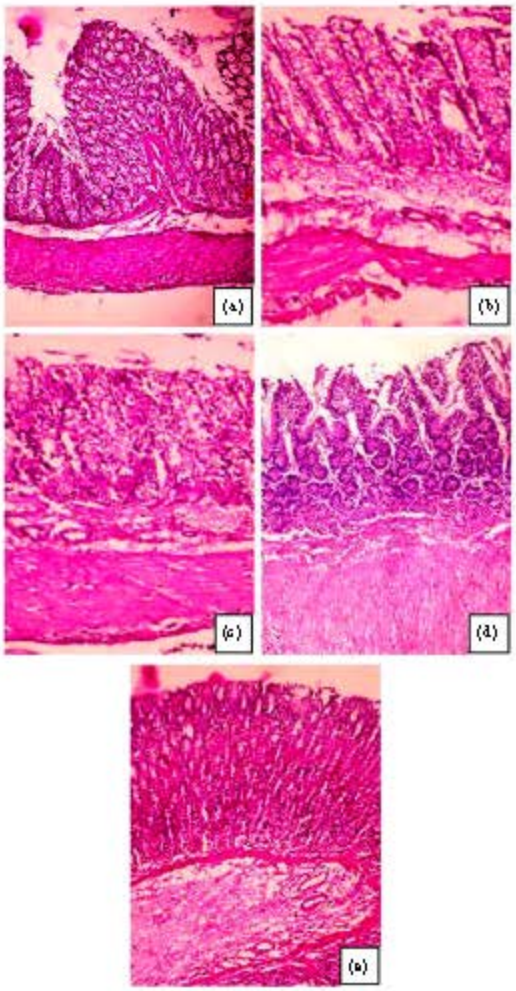

Fig. 2: Histopathologic photographs of ileum. (a) Normal ileum of rat, (b) Inflamed ileum of rat showing submucosa with severe infiltration and edema, (c, d) Ileum of MEO treated (100 and 200 mg kg^{-1}, respectively) rat showing recovered submucosal edema and few inflammatory cells, (e) Sulfasalazine treated rat showing recovered submucosal edema. (H and E × 20)

Histopathological Results

The histopathological examinations of indomethacin treated group showed advanced lesions as necrosis. The MEO treated group showed reduced intensity of lesions without any evidence of necrosis and regeneration or inflammatory reaction. Treatment of rat with sulfasalazine showed suppressed inflammatory reaction (Fig. 2a-e, 3a-e).

Indomethacin, a non-selective COX inhibitor produces enterocolitis in rats which is characterized by linear ulceration, thickening and transmural inflammation. The mechanism of ID induced enterocolitis have not been fully illustrated, but in earlier studies it suggested that, inhibition of protective prostaglandins PGE_1, PGE_2 and prostacyline (PGI_2) may be one of the mechanism by which ID induces injury, in addition with bacteria and bacterial products

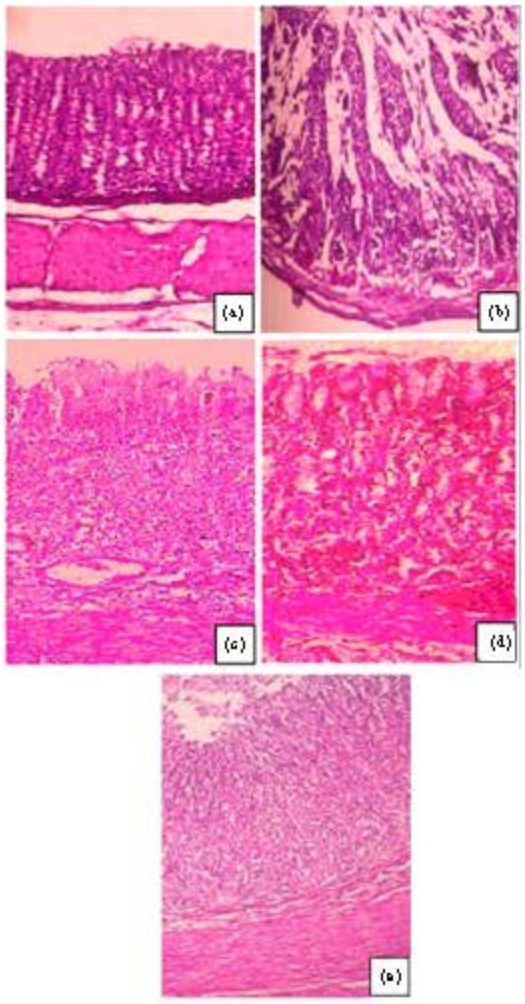

Fig. 3: Histopathological photograph of colon. (a) Normal colon mucosa of rat, (b) Indomethacin-treated rats showing disorganized epithelial layer and diffuse inflammatory leukocytic infiltration, (c, d) Colonic sections from rats treated orally with MEO (100 and 200 mg kg^{-1}, respectively) showing attenuation of the morphological disturbance and reduction of the inflammatory cell infiltration and mucosal edema associated with indomethacin administration, (e) Sulfasalazine (500 mg kg^{-1}) attenuated the extent and severity of the histological signs of cell damage. (H and E × 20)

(Sharon and Stenson, 1985). The inhibition of cyclooxygenase and thereby inhibition of protective prostaglandins may also be a major factor in the pathogenesis of enterocolitis. More recent studies have shown that neutrophils and neutrophil derived oxidants are involved in the pathogenesis of indomethacin induced acute gastropathy. Other studies also suggested that biliary secretion, food intake, luminal bacteria and bacterial cell wall polymers also causes pathogenesis of indomethacin induced intestinal inflammation.

In consistent with findings of previous report (Jagtap *et al*., 2004), in present study administration of indomethacin leads to develop acute intestinal inflammation, manifested by a thickening of the bowel wall, mesenteric hemorrhage, mesentery adhesion and multiple mucosal ulcers of small intestine and colon. The MEO treatment showed reduced intensity of lesions without any evidence of necrosis and inflammatory reaction in both ileum and colon.

A significant increase in serum LDH level was observed in colitis induced animals is consistent with findings of earlier reported by Hager *et al*. (2007). The MEO treatment decreased serum LDH level in dose dependent manner.

The earlier reports suggest that, herbal plants like *Boswellia serrata* (Krieglstein *et al*., 2001) and polyherbal formulation containing *Aegle marmeloes*, *Coriandrum sativum*, *Cyperus rotundus* and *Vetiveria zinzanioids* (Jagtap *et al*., 2004) have shown the effect on indomethacin induced enterocolitis, due to its anti-inflammatory and antimicrobial property. It is well known that methanolic extract of MEO have shown antiinflammatory and antimicrobial property (Ahmad *et al*., 1998). This gives an idea that protective effect of methanolic extract on indomethacin induced enterocolitis may be due to its antiinflammatory and anti microbial property.

CONCLUSION

The present data suggest that the *Emblica officinalis* can protect indomethacin induced enterocolitis in rats and may be beneficial in patients with inflammatory bowel diseases. This protective effect may, at least in part, be due to their anti-inflammatory and/or antioxidant actions. However, more detailed studies are essential to identify exact mechanism of action. Suitable clinical examination is also necessary to confirm this activity in human disease.

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