Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Issue 1, 2012

Research Article

ANTI-INFLAMMATORY EFFECT OF *DAUCUS CAROTA* ROOT ON EXPERIMENTAL COLITIS IN RATS

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Received: 29 Aug 2011, Revised and Accepted: 25 Nov 2011

ABSTRACT

Inflammatory bowel disease (IBD) is a chronic disease of unknown etiology which is characterized by chronic and spontaneously relapsing inflammation. The aim of present investigation was to study the anti-inflammatory effect of aqueous extract of *Daucus carota* (AEDC) in acetic acid induced experimental colitis in wistar rats. The animals were received 7 days pretreatment with *Daucus carota* (100, 200 and 400 mg/kg, p.o.) or vehicle (distilled water, 1 ml) before the induction of colitis. Colitis was induced by intrarectal instillation of 2 ml (4% v/v) acetic acid solution. Intrarectal instillation of acetic acid resulted significant decreased in food and water intake, body weight of animals. It caused enhanced colon weight, colon width, colon weight to length ratio, spleen weight, ulcer area, ulcer index, colonic myeloperoxidase (MPO) and nitric oxide. Pretreatment with *Daucus carota* aqueous extract (200 and 400 mg/kg, p.o.) for 7 days significantly ($P < 0.01$ and $P < 0.001$ respectively) and dose dependently attenuated these decreased food and water intake, body weight. The 7 days pretreatment with *Daucus carota* aqueous extract (200 and 400 mg/kg, p.o.) significantly decreased stool consistency, macroscopical score, colon weight, colon width, colon weight to length ratio, spleen weight, ulcer area, ulcer index, colonic MPO and nitric oxide. It significantly attenuated histological alterations associated with acetic acid induced ulcerative colitis. The present investigation elucidates anti-inflammatory effect of aqueous extract of *Daucus carota* root in acetic acid induced experimental colitis by inhibition of release of oxido-inflammatory mediators such as MPO and nitric oxide.

Keywords: Acetic acid, *Daucus carota*, Inflammatory Bowel Disease, Myeloperoxidase, Nitric oxide, Oxidative stress, Ulcerative colitis

INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic inflammatory, idiopathic disorder of intestine with unknown etiology. Inflammatory bowel disease is of two different types; ulcerative colitis and Crohn's disease. Ulcerative colitis mainly involves inflammation of colon where as Crohn's disease is an inflammation of gastrointestinal tract from the mouth to the anus. Only 10 to 20% of people suffering from IBD have major problem that this disease itself leads to colon cancer or bowel cancer.[1, 2] Generation of the free radicals during the progression of the IBD has been suggested as an important factor for initiation and progression of cancer.[3] Genetic modulation, infective agents, immunological disturbance, smoking, microorganism were found to be responsible for mucosal inflammation, hemorrhage and development of ulcers in colon.[4-6]

Cytokines like TNF-α, IL-1β, IL-6 and IL-8 released form macrophages were found to be responsible for generation of reactive oxygen species (ROS) which activates the oxidative stress responsive gene that plays a vital role in development and maintenance of IBD.[7- 9] The over production of ROS caused destruction of mucosal epithelial layer and thus lead to impairment in experimental colitis.[10]

People age between 15 to 35 years old mainly suffered from IBD which continues for lifetime. Intrarectal instillation of acetic acid in laboratory animals mimics similar pathobiological condition as that of human. Hence, acetic acid induced IBD is a reliable and reproducible animal model and is useful for screening of drugs for ulcerative colitis.[11-13] Acetic acid induced colitis was associated with the elevated level of oxidation and lipid peroxidation which caused alterations in mucosal antioxidant defenses system.[14]

Synthetic chemical moieties like 5-amino salicylate, corticosteroids, antimicrobials and immunosuppressive agents such as azathioprine and mercaptopurine, etc. with antioxidant potential are the present treatment regimens. But, their disadvantages like high relapse rate and wide range of side effects limits their utility in treatment of IBD.[15]

Polyphenols, flavonoids possess the potential for the treatment of chronic inflammation in experimental model of IBD.[16] Flavonoids has free radical scavenging ability along with the inhibitory potential for the proinflammatory cytokines.[17]

Daucus carota plant has been used as a diuretic and inotropic, cystitis, gout and lithuria in the traditional system of medicine.[18, 19] It has been evaluated for a wide spectrum of activity like analgesic and anti-inflammatory,[20] hepatoprotective,[21] hypoglycemic,[22] antiulcer,[23] antifertility,[24] anticancer,[25] anti-tumor.[26]

The objective of present investigation was to unravel therapeutic potential of *Daucus carota* in acetic acid induced experimental colitis in laboratory animals by assessing various macroscopic, microscopic and biochemical parameter.

MATERIALS AND METHODS

Collection of plant material

The roots of *Daucus carota* L. were collected from rural areas of Pune district, Maharashtra in the month of October 2010. Authentication of Plant was carried out by P.G. Diwakar, Joint Director, Botanical Survey of India, Pune.

Preparation of extract

The fresh roots (1 kg) of Daucus carota were peeled, washed, cut into small pieces and homogenized in blender without adding water. The homogenate roots were squeezed and filtered through a cheese cloth to yield a residue. The obtained juice was lyophilized to get in powdered form. The powdered extract was stored in deep freezer at -20^{0}C for experimental use.

Preliminary phytochemical screening

The Preliminary phytochemical screening of the above aqueous extract of *Daucus carota* (AEDC) L. was carried out according to the methods described by Khandelwal *et al.*; Kokate *et al.*[27, 28] Phytochemical analysis of the extract was performed for the identification of phytochemical like alkaloid, flavonoids, steroid & phenols etc.

Animals

Healthy adult male swiss albino mice (20-30 g) and male wistar rats (230-250 g) were obtained from the National Toxicological Centre, Pune (India). The animals were housed in groups of 6 in solid bottom polypropylene cages. They were maintained at 24^{0}C ± 1^{0}C, with relative humidity of 45-55% and 12:12 h dark/light cycle. The animals were acclimatized for a period of two weeks and were kept

under pathogen free conditions. The animals had free access to standard pellet chow (Chakan Oil Mills, Sangli) throughout the experimental protocol, with the exception of overnight fasting before induction of experimental colitis. The animals had access to filtered water. The pharmacology and acute toxicity protocols were approved by the Institutional Animal Ethics Committee (IAEC).

Drugs and chemicals

Prednisolone was obtained as a gift sample from Symed Pharmaceuticals Pvt. Ltd., Hyderabad. Acetic acid, anaesthetic ether, ethanol, formalin, chloroform, ether, hydrochloric acid and conc. Sulphuric acid were purchased from S.D. Fine Chemicals, Mumbai, India. Sulphanilamides, naphthalamine diamine HCl, phosphoric acid were obtained from LobaChemi Pvt. Ltd., Mumbai, India.

Acute toxicity testing

Acute oral toxicity studies in swiss albino mice were performed according to OECD guidelines using AOT 425 software. Graded doses of the *Daucus carota* were dissolved in distilled water were administered orally and the animals were observed for 2 weeks following administration. Body weight, food consumption, fluid intake and psycho-motor activities were recorded daily.

Dosages of *Daucus carota* extract and standard drugs used

The Freshly prepared aqueous solution of *Daucus carota* was administered to animals orally for 7 days in three different dosages (100 mg/kg, 200 mg/kg and 400 mg/kg). On 8th day, the colitis was induced by intrarectal administration of acetic acid. The drug treatment was continued even after administration of acetic acid. Prednisolone was used as standard drug. Prednisolone was not given as pre-treatment. Prednisolone was administered at a dose of 2 mg/kg/day orally in rats as suspension in 0.5% of sodium CMC.

Induction of colitis

Colonic inflammation was induced in fasted rats following the method of Millar *et al.*[14] The study comprised of six groups of six animals each as follows:

Group I: Normal animals (received 2 mg/kg/day of distilled water)
Group II: Acetic acid control animals (received 2 ml of 4% acetic acid solution intrarectally on 8th day)
Group III: AEDC (100 mg/kg) treated animals (received 7 days pretreatment with 100 mg/kg of AEDC, p.o. and 2 ml of 4% acetic acid solution, intrarectally on 8th day. Drug treatment was continued till 11th day
Group IV: AEDC (200 mg/kg) treated animals (received 7 days pretreatment with 200 mg/kg of AEDC, p.o. and 2 ml of 4% acetic acid solution, intrarectally on 8th day. Drug treatment was continued till 11th day
Group V: AEDC (400 mg/kg) treated animals (received 7 days pretreatment with 400 mg/kg of AEDC, p.o. and 2 ml of 4% acetic acid solution, intrarectally on 8th day. Drug treatment was continued till 11th day
Group VI: Prednisolone treated group, which received Prednisolone (2 mg/kg, p.o., for 3 days) and acetic acid (2 ml of 4% solution, once, intrarectally). Prednisolone and acetic acid acetic acid treatment was started on the same day.

On the 11th day blood was withdraw by retro orbital puncture and animals were sacrificed by cervical dislocation. Colons were collected and spleen from each animal was weighed. Portions of colonic specimens were kept in 10% formalin for histopathological studies.

Evaluation of the disease

The disease induced in experimental animals was evaluated based on its macroscopic characteristics.[29]

Evaluation based on macroscopic characters

Pieces of rat colon (10 cm long each) were scored for macroscopic features using scoring pattern. (Table 1)

Table 1: Colonic macroscopic scoring pattern for evaluation of disease activity index

Score	Macroscopic changes
0	No visible change
1	Hyperemia at sites
2	Lesions having diameter l mm or less
3	Lesions having diameter 2 mm or less (number < 5)
4	Lesions having diameter 2 mm or less (number 5–10)
5	Lesions having diameter 2 mm or less (number > 10)
6	Lesions having diameter more than 2 mm (number < 5)
7	Lesions having diameter more than 2 mm (number 5–10)
8	Lesions having diameter more than 2 mm (number > 10)

Determination of ulcer area and ulcer index

For determination of ulcer area, each colon was incised and washed with normal saline and scanned using CCD scanner at a magnification of 2400 dpi. The images were processed using image J software and adobe Photoshop to determine ulcer area. The ulcer index and ulcer index was determined as per the method described by Dengiz *et al.*[30]

Biochemical assays

500 mg tissue from the colon was excised, washed, chopped and homogenized at 3000 rpm in chilled Tris buffer (10 mM, pH 7.4) at a concentration of 10% w/v. The homogenates were centrifuged at 10,000 g at 0^0 C for 20 min, to obtain supernatant volume of 4 ml. It was divided into aliquot to determined myeloperoxidase (MPO content) (2.0 ml), nitric oxide (NO) level (0.5 ml)

Determination of colonic MPO contents

The colonic myeloperoxidase assay was assessed as a marker of neutrophil infiltration according to the method described by Krawisz *et al.*[31] Myeloperoxidase activity was defined as the quantity of enzyme degrading 1 µmol of peroxide per min at 25^{0}C and was expressed in units per gram (U/gm) of wet scrapings.

Determination of colonic nitrite/nitrate level

Colonic NO level was estimated as nitrite and nitrate by acidic Griess reaction after reduction of nitrate to nitrite by vanadium trichloride according to the method described by Miranda *et al.*[32] The Griess reaction relies on a simple colorimetric reaction between nitrite, sulfonamide and N-(1-naphthyl) ethylenediamine to produce a pink azo-product with maximum absorbance at 543 nm. The concentrations were determined using a standard curve of sodium nitrate and the results were expressed as µg/mg of wet tissue.

Histopathological studies

Freshly excised colon of one animal from each group was washed with saline and preserved in 10% formaldehyde solution for histopathological studies. It was processed for 12 hr. using isopropyl alcohol, xylene and paraffin embedded for light microscopic study. Paraffin embedded tissue section cut at 5µm thickness were prepared and stained after deparaffination using hematoxylene and eosin stain (H & E) to verify morphological assessment of colon damage. Photomicrographs were captured at a magnification of 40 X.

Data and statistical analysis

All the results were expressed as mean ± S.E.M. Data analysis was performed using GraphPad Prism 5.0 software (GraphPad, San Diego, USA). Statistical comparisons were made between drug-treated groups and colitis control animals. Data of biochemical parameters were analyzed using one-way ANOVA; Dunnett's multiple range test was applied for post hoc analysis. A value of $P < 0.05$ was considered to be statistically significant.

RESULTS

Acute Toxicity Testing

Acute toxicity studies of AEDC shows no signs and symptoms such as restlessness, respiratory distress, diarrhea, convulsions and coma and it was found safe up to 5000 mg/kg.

Preliminary phytochemical screening

AEDC L. root was screened for various chemical tests as per the reported methods [27, 28] and was found to contain alkaloids, flavonoids, tannins, saponins and cardiac glycosides. (Table 2)

Acetic acid-induced colitis

Intrarectal instillation of 4% acetic acid resulted in colonic inflammation. Pretreatment with AEDC showed suppressed inflammatory reaction.

Effect of AEDC on foot intake, water intake and body weight

As compared to normal rats, the food intake, water intake and body weight in the acetic acid control rats was significantly decreased ($P < 0.001$). Pretreatment with AEDC (200 and 400 mg/kg) for 7 days significantly and dose dependently ($P < 0.01$ and $P < 0.001$, respectively) increased the food intake, water intake and body weight of rats. (Table 3)

Effect of AEDC on stool consistency

The stool consistency of all the animals was observed for 11 days and scoring has been done from 0-4 on the basis of consistency of stools. Intrarectal instillation of 4% acetic acid resulted significant increased ($P < 0.001$) in stool consistency in acetic acid control rats as compared to normal rats. Pretreatment with AEDC (400 mg/kg) for 7 days significantly ($P < 0.01$) decreased stool consistency as compared to acetic acid control rats. (Table 3)

Effect of AEDC on macroscopical score

After intrarectal instillation of 4% acetic acid, the colons were examined for signs of hemorrhage and ulceration by an independent blind observer. The mean microscopical score in acetic acid control rats was significantly increased ($P < 0.01$) as compared to normal rats. The 7 days pretreatment with AEDC (200 and 400 mg/kg) significantly and dose dependently ($P < 0.01$ and $P < 0.001$, respectively) decreased these macroscopical lesions produced by the intrarectal instillation of acetic acid. (Table 3)

Effect of AEDC on colon weight and colon width

Intrarectal instillation of acetic acid resulted in significant increased ($P < 0.01$) in colon weight and colon width in acetic acid control rats as compared to normal rats. Pretreatment with AEDC (200 and 400 mg/kg) for 7 days significantly and dose dependently ($P < 0.01$ and $P < 0.001$, respectively) attenuated these increased in colon weight and colon width as compared to acetic acid control rats. (Table 4)

Effect of AEDC on colon weight to length ratio

When compared with normal rats, the ratio of colon weight/length was found to be increased significantly ($P < 0.001$) in acetic acid control rats after intrarectal instillation of acetic acid. Pretreatment with AEDC (400 mg/kg) for 7 days significantly ($P < 0.05$) decreased the colon weight/length ratio as compared to acetic acid control rats. (Table 4)

Effect of AEDC on spleen weight

Intrarectal instillation of acetic acid was associated with splenic enlargement. Spleen weight in acetic acid control rats was significantly increased ($P < 0.001$) as compared to normal rats. The 7 days pretreatment with AEDC (200 and 400 mg/kg) significantly and dose dependently ($P < 0.01$ and $P < 0.001$, respectively) decreased spleen weight as compared to acetic acid control rats. (Table 4)

Effect of AEDC on ulcer area and ulcer index

Ulcer area and ulcer index in acetic acid control rats was significantly increased ($P < 0.001$) after intrarectal instillation of 4% acetic acid when compared with normal rats. Pretreatment with AEDC (200 and 400 mg/kg) for 7 days significantly and dose dependently ($P < 0.01$ and $P < 0.001$, respectively) attenuated these increased ulcer area and ulcer index as compared to acetic acid control rats. (Table 4)

Effect of AEDC on colonic MPO contents

The MPO content in acetic acid control rats was significantly increased ($P < 0.001$) after intrarectal instillation of 4% acetic acid as compared to normal rats. The 7 days pretreatment with AEDC (200 and 400 mg/kg) significantly and dose dependently ($P < 0.01$ and $P < 0.001$, respectively) attenuated these elevated level of MPO as compared to acetic acid control rats. (Figure 1)

Effect of AEDC on colonic nitrite level

Intrarectal instillation of acetic acid resulted significant increased ($P < 0.001$) in colonic nitrite level as compared to normal rats. Pretreatment with AEDC (200 and 400 mg/kg) for 7 days significantly and dose dependently ($P < 0.05$ and $P < 0.01$, respectively) decreased the colonic nitrite level as compared to acetic acid control rats. (Figure 2)

Effect of AEDC on histopathology of colon

Histopathological damage was evaluated in colonic samples stained with hematoxylin and eosin. In the epithelial crypts of the mucosal layer were remains intact. There was no infiltration of inflammatory cells (Figure 3A).The intrarectal instillation of acetic acid resulted in significant development of transmural necrosis, submucosal edema, ulceration along with cellular infiltration.

The epithelial crypts were completely lost (Figure 3B). In AEDC (400 mg/kg) pretreated rats (Figure 3C) and prednisolone (2mg/kg) treated rats (Figure 3D) epithelial crypts were decreased. It significantly attenuated the extent and severity of the histological signs of cell damage that were associated with intrarectal instillation of acetic acid. (Table 5)

Table 2: Preliminary phytochemical constituents present in aqueous extract of *Daucus carota* L. root

Sr. No.	Phytoconstituent	Present/ Absent
1.	Alkaloids	
	Mayer's reagent	+
	Wagner's reagent	+
	Dragendorff's reagent	-
2.	Tannin	+
3.	Saponin	+
4.	Flavonoids	+
5.	Steroids	-
6.	Cardiac glycosides	+
7.	Carbohydrate	-
8.	Terpenoid	-

+ Present, - Absent

Table 3: Effect of AEDC on food intake, water intake, percent decrease in body weight, stool consistency and macroscopic score of rat in acetic acid induced IBD.

Parameter	Normal	Acetic acid Control	Prednisolone (2 mg/kg)	*Daucus carota*		
				100 mg/kg	200 mg/kg	400 mg/kg
Food intake	151.1 ± 4.27	79.30 ± 7.26###	137.0 ± 6.81	85.80 ± 4.93	116.2 ± 5.82**	129.6 ± 7.85***
Water intake	178.4 ± 6.40	104.0 ± 6.96###	163.0 ± 4.23	123.6 ± 4.63	133.6 ± 8.54**	161.2 ± 4.64***
% Decrease in body weight	-5.82 ± 0.53	6.88 ± 0.59###	-2.14 ± 1.22	4.14 ± 0.92	-1.34 ± 0.65***	-2.12 ± 1.09***
Stool consistency	0.20 ± 0.20	3.60 ± 0.24###	1.60 ± 0.50	3.20 ± 0.37	2.40 ± 0.24	1.80 ± 0.20**
Macroscopical score	0.00 ± 0.00	7.20 ± 0.37###	2.40 ± 0.50	6.20 ± 0.37	4.60 ± 0.50**	3.20 ± 0.58***

Data are expressed as mean ± S.E.M. from five rats and analyze by one way ANOVA followed by Dunnett's test. **P* < 0.05, ***P* < 0.01, ****P* < 0.001 as compared to acetic acid control group

Table 4: Effect of AEDC on colon weight, colon width, colon weight to length ratio, spleen weight, ulcer area and ulcer index of rat in acetic acid induced IBD.

Parameter	Normal	Acetic acid Control	Prednisolone (2 mg/kg)	*Daucus carota*		
				100 mg/kg	200 mg/kg	400 mg/kg
Colon weight	1.23 ± 0.11	2.63 ± 0.13###	1.33 ± 0.07	2.43 ± 0.13	1.89 ± 0.11**	1.51 ± 0.12***
Colon width	0.45 ± 0.04	1.82 ± 0.08###	0.71 ± 0.10	1.64 ± 0.10	1.29 ± 0.10**	1.03 ± 0.08***
Colon weight to length ratio	0.16 ± 0.01	0.28 ± 0.02###	0.17 ± 0.01	0.28 ± 0.01	0.25 ± 0.01	0.21 ± 0.02*
Spleen weight	1.33 ± 0.09	2.51 ± 0.10 ###	1.52 ± 0.08	2.38 ± 0.13	2.02 ± 0.12**	1.78 ± 0.12***
Ulcer area	0.00 ± 0.00	30.76 ± 1.15###	4.86 ± 0.88	25.51 ± 1.65*	20.74 ± 1.28**	12.74 ± 1.38***
Ulcer index	0.00 ± 0.00	58.37 ± 2.15###	14.13 ± 1.38	50.91 ± 1.99*	39.81 ± 2.13**	25.79 ± 2.66***

Data are expressed as mean ± S.E.M. from five rats and analyze by one way ANOVA followed by Dunnett's test. **P* < 0.05, ***P* < 0.01, ****P* < 0.001 as compared to acetic acid control group

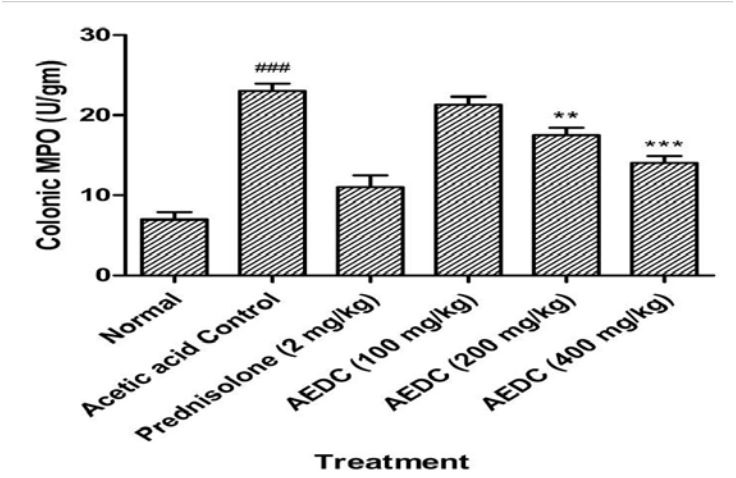

Fig. 1: Effect of AEDC on colonic MPO concentrations in acetic acid induced IBD. Data are expressed as mean ± S.E.M. from five rats and analyze by one way ANOVA followed by Dunnett's test. **P* < 0.05, ***P* < 0.01, ****P* < 0.001 as compared to acetic acid control group

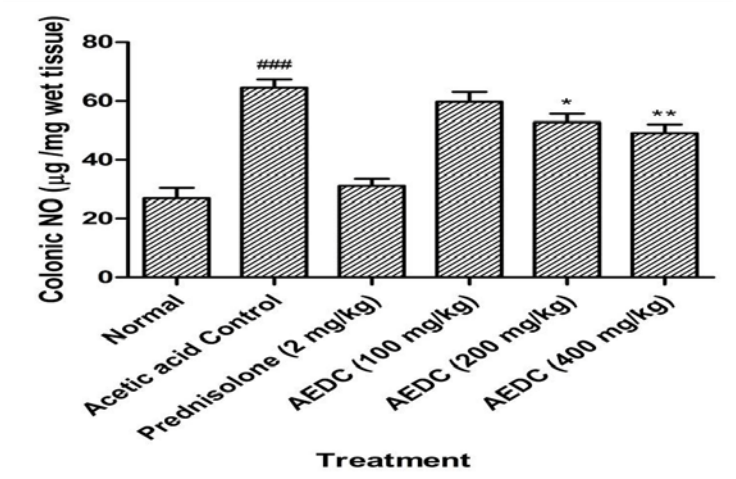

Fig. 2: Effect of AEDC on colonic nitrite level in acetic acid induced IBD. Data are expressed as mean ± S.E.M. from five rats and analyze by one way ANOVA followed by Dunnett's test. **P* < 0.05, ***P* < 0.01, ****P* < 0.001 as compared to acetic acid control group

Table 5: Effect of AEDC pathological changes of rat colon in acetic acid induced IBD.

Group	Ulceration	Hyperemia	Necrosis	Edema	cellular in filtration	Goblet cell hyperplasia
Normal	0	+	0	+	0	0
Acetic acid control	++++	++++	++++	+++	++++	++
Prednisolone (2 mg/kg)	+	+	++	+	+	++
AEDC (400 mg/kg)	++	+	+	++	++	++

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 75 %

++++: damage/ active changes up to more than 75 %

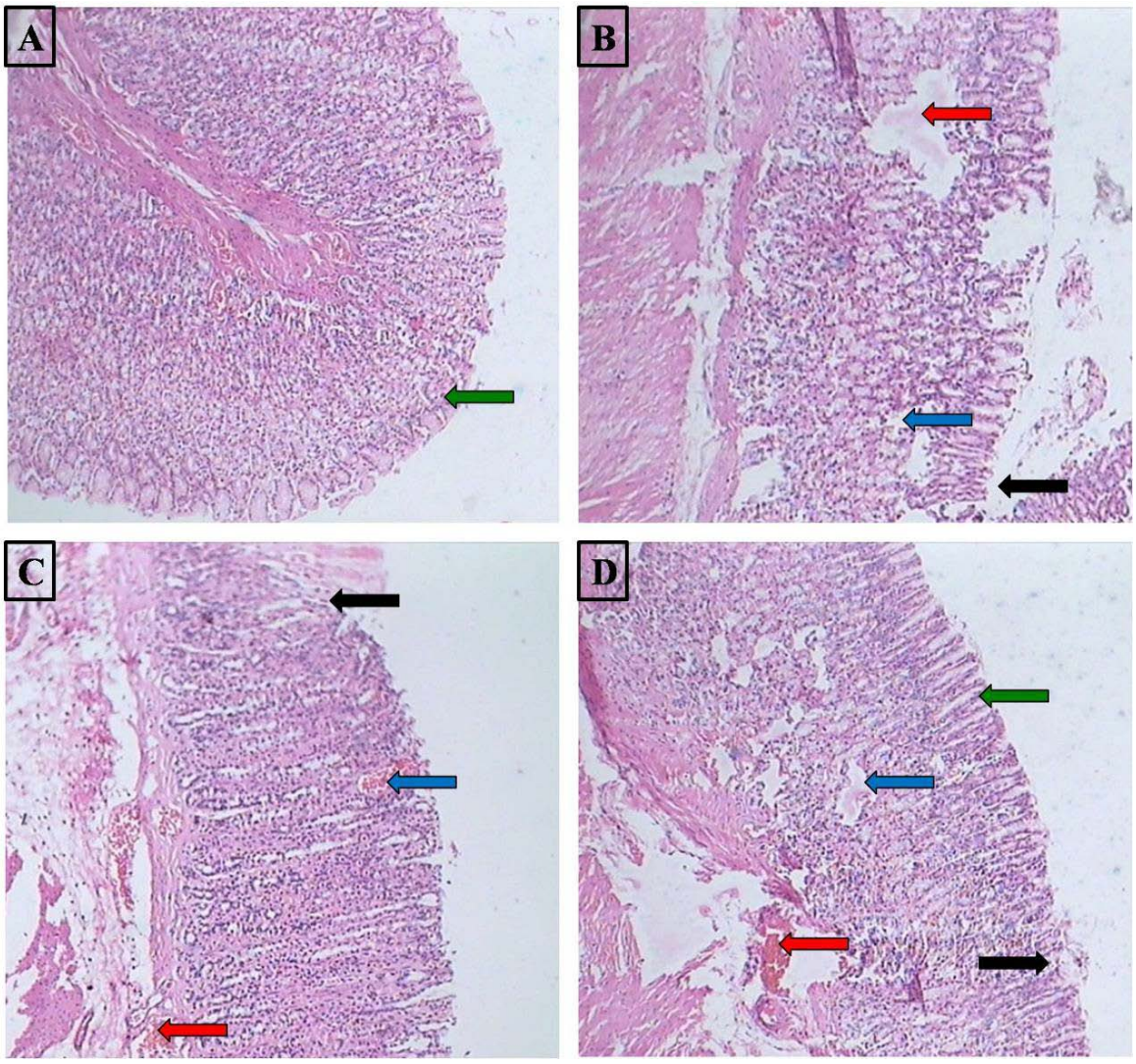

Fig. 3: Photomicrographs of sections of colons from rats stained with H&E

Colon microscopic image of (A) Normal rat with intact epithelial and mucosal layer (green arrow); (B) Acetic acid induced colitis rat with extensive damage including edema in submucosa (red arrow) and cellular infiltration (blue arrow), necrosis (black arrow) and ulceration; (C) Prednisolone (2 mg/kg, p.o.) treated rat with cellular infiltration (blue arrow), necrosis (black arrow) and hemorrhages (red arrow); (D) *Daucus carota* (400 mg/kg p.o.) 7 days pretreated rat with edema in submucosa (green arrow), cellular infiltration (blue arrow), necrosis (black arrow) and hemorrhages (red arrow). Images (40 X magnification) are typical and representative of each study group.

DISCUSSION

Inflammatory bowel disease (IBD) is chronic relapsing conditions characterized by up-regulated pro inflammatory mediators and dysregulated immune responses resulting in tissue damage. The genetics, immunology and environment are the multiple etiologic theories which are related with IBD. [33]

Acetic acid induced experimental colitis is one of standardized model of IBD. An array of factor responsible for the induction and maintenance of the colitis includes enhanced vasopermeability,

prolonged neutrophils infiltration and elevated levels of inflammatory mediators. [34]

The present investigation demonstrated that acetic acid-induced ulcerative colitis was associated with macroscopic, microscopic and biochemical changes. The intrarectal instillation of acetic acid resulted massive localized erosion of the colonic mucosa leading to sever localized inflammation and hemorrhages.[35, 36]

Increased weight of colon as well as width of colon along with elevated weight/length ratio is reflecting the degree of local inflammation along with the other parameters of edema and wall thickening. The weight of the colon tissue is elevated due to inflammatory response which is indicative of severity and extent of the disease.[37] The *Daucus carota* significantly decreased not only the wet weight of distal colon segments but also the colon damage score. Further, it effectively reduced the histological signs of inflammation such as leukocyte infiltration, edema and tissue injury by virtue of its healing property.

Acetic acid induced colitis was associated with splenic enlargement. As spleen destroys unnecessary red blood cells and holds a reservoir of blood. Spleen is an essential part of the immune system and reticuloendothelial system.[38] *Daucus carota* significantly decreased the splenic enlargement via its immunomodulatory potential.

Ulcer area is reflecting the degree of the gross morphological lesions as well as necrotic area of various sizes. Ulcer area and ulcer index were quantitatively determined. Ulcer area significantly decreased in pretreatment with *Daucus carota* depicting its microflora protective activity from the corrosive effect of acetic acid.

Myeloperoxidase is a marker of neutrophil infiltration. Elevated level of neutrophil resulted in production of superoxide anion and generation of vicious cycle that would contribute efficiently to the tissue necrosis and mucosal dysfunction.[39, 40] *Daucus carota* significantly attenuated this elevated level of MPO by virtue of its antioxidant property.

Nitric oxide (NO) is an unconventional intracellular messenger playing a vital role in various pathological and physiological processes. NO is an oxidant as it reacts with reactive oxygen species (ROS). It results in cellular damage as the oxidation process is not specific. It also forms peroxynitrite anion ($ONOO^-$) by reacting with ROS which is enough to avoid the action of antioxidant system.[41] Pretreatment with *Daucus carota* restores the elevated level of colonic nitric oxide.

Presently prednisolone is a treatment regimen for IBD. However, the side effects of prednisolone like stress, blurred vision, anxiety, hepatic steatosis, depression, restlessness are well documented in literature.[42] Hence, it can't be prescribed to geriatric patients. However, *Daucus carota* did not exhibit such side effects and thus provides a ray of hope for these patients.

It have been documented that *Daucus carota* is contain high source of vitamins like B_1, B_2, C, D_2, E, nicotinic acid, flavonoids,[43, 44] β-carotene, α-carotene, γ-carotene, lycopene, cryptoxanthin, leutenin, many partly degraded carotenoids such as abscisic acid, trisporic acid, β-apo-carotenoids like violaxanthin. [45, 46]

CONCLUSION

From the present investigation it can be deduced that *Daucus carota* possesses potent activity against various pathological changes caused by administration of acetic acid. The flavonoids present in *Daucus carota* may possess anti-inflammatory potential against acetic acid induced experimental colitis by inhibition of release of oxido-inflammatory mediators such as MPO and nitric oxide.

ACKNOWLEDGEMENTS

The authors would to like acknowledge Prof. R. D. Patankar, Principal, Abhinav College of Pharmacy and Dr. S. L. Bodhankar, Head and Professor, Department of Pharmacology, Poona College of Pharmacy, Bharati Vidyapeeth Deemed University, Pune, India for their constant support and valuable suggestions for the research work. We are also thankful to Dr. Vasant Narke, Director, TOXINDIA, Pune, India for providing necessary facilities to carry out the study.

REFERENCES

1. Mclllmurray M.B. and Langman M.J.S.: Large bowel cancer: causation and management. Gut 1975; 16(10): 815-20.
2. Qureshi A.A., Omer S.K. Eswar K. and Bhajipale N.S.: Probiotics in diarrhea: myths and facts. Int. J. Pharm. Pharm. Sci. 2010; 2(3): 23-28.
3. Mallet S.J., Lennard-Jones J.E., Bomgleu J. and Gilon E.: Living with the disease. Lancet 1978; 2: 619-21.
4. *Dipiro* J.T. and *Schade* R.R.: Inflammatory bowel disease. In: Dipiro J, Talbert R, editors. Pharmacotherapy: A Pathophysiologic Approach. 4th ed., Connecticut, 1999. p. 571-85.
5. Berardi R.R.: Inflammatory bowel disease. In: Herfindal E, Gourley D, editors. Textbook of Therapeutics-Drug and Disease Management. 7th ed. Lippincott Williams & Willkins, 2000. p. 483-502.
6. Kumari N. and Deshwal R.K.: Antioxidants and their protective action against DNA damage. Int. J. Pharm. Pharm. Sci. 2011; 3(4): 28-32.
7. Seo H.G., Takata L., Nakamura M., Tatsumi H., Fujii J. and Taniguchi N.: Induction of nitric oxide synthase and concomitant suppression of superoxide dismutases in experimental colitis in rats. Arch. Biochem. Biophys. 1995; 324: 41-7.
8. Larrick J.W. and Wright S.: Cytotoxic mechanism of tumor necrosis factor-α. FASEB J. 1990; 4: 3215-23.
9. Nagore D.H., Ghosh V.K., Patil M.J. and Wahile A.M.: In vitro antioxidant and in vivo anti-inflammatory activity of *Cassia sophera* Linn. Int. J. Pharm. Pharm. Sci. 2010; 2(1): 113-121.
10. Oz H.S., Chen T.S., McClain C.J. and de Villiers W.J.: Antioxidants as novel therapy in a murine model of colitis. J. Nutr. Biochem. 2005; 16: 297-304.
11. Boismenu R. and Chen Y.: Insights from mouse models of colitis. J. Leukoc. Biol. 2000; 67: 267-78.
12. Shanahan F.: Inflammatory bowel disease: immunodiagnostics, immunotherapeutics and ecotherapeutics. Gastroenterol. 2001; 120: 622-35.
13. Strober W., Fuss I.J. and Blumberg R.S.: The immunology of mucosal models of inflammation. Annu. Rev. Immunol. 2002; 20: 495-549.
14. Millar A.D., Rampton D.S. and Chander C.L. and *et al.*: Evaluating the antioxidant potential of new treatments for inflammatory bowel disease using a rat model of colitis. Gut 1996; 39(3): 407-15.
15. Joshi R., Kumar S., Unnikrishnan M. and Mukherjee T.: Free radical scavenging reactions of sulfasalazine, 5-aminosalicylic acid and sulfapyridine: mechanistic aspects and antioxidant activity. Free Radic. Res. 2005; 39: 1163-72.
16. Havsteen B.: Flavonoids, a class of natural products of high pharmacological potency. Biochem. Pharmacol. 1983; 32(7): 1141-8.
17. Mora A., Paya M., Rio J.L. and Alcaraz M.J.: Structure-activity relationships of polymethoxyflavones and other flavonoids as inhibitors of non-enzymic lipid peroxidation. Biochem. Pharmacol. 1990; 40(4): 793-7.
18. Barnes J., Anderson L.A. and Phillipson J.D.: Editors, Herbal medicine. 3rd ed. Pharmaceutical Press, London, 2002, 593-5.
19. Thomas K.J., Nicholl J.P. and Coleman P.: Use and Expenditure on Complementary Medicine in England: A population based survey. Complement Ther. Med. 2001; 9(1): 2-11.
20. Prochezhian E. and Ansari S.H.: Analgesic and anti-inflammatory activity of volatile oil from *Daucus carota* Linn. Indian J. Nat. Prod. 2000; 16: 24-6.
21. Bishayee A., Sarkar A. and Chaterjee M.: Hepatoprotective activity of carrot against carbon tetrachloride intoxication in mouse liver. J. Ethanopharmacol. 1995; 47: 69-74.
22. Neef H., Declercq P. and Laekeman G.: Hypoglycaemic activity of selected European plants. Phytother. Res. 1995; 9: 45-8.
23. Wehbe K., Mroueh M. and Daher C.F.: The Potential Role of *Daucus carota* Aqueous and Methanolic Extracts on

Inflammation and Gastric Ulcers in Rats. J. Comple. Integ. Med. 2009; 6(1): 7.

24. Prakash A.O.: Biological Evaluation of some medicinal plant extracts for contraceptive efficacy. Contracept Deliv. Sys. 1984; 5: 9.
25. Diab-Assaf M., Mroueh M. and El-Sharif S.: Evaluation of anti-cancer effect of *Daucus carota* on the human promyelocytic leukemia HL-60 cells. AACR International Conference on Molecular Diagnostics in Cancer Therapeutic Development, Atlanta, USA, 2007.
26. Abou Zeinab R.M., Mroueh M. and Daher C.F.: Potent anti-tumor promoting effects of *Daucus carota* oil extract in mice. Planta Medica 2008; 74: 1008.
27. Khandelwal K.R.: Practical Pharmacognosy, Technique and Experiments. 8th edition. Pune: Nirali Prakashan; 2007. p. 149-53.
28. Kokate C.K. Practical Pharmacognosy. New Delhi: Vallabh Prakashan, 2005. p. 107-8, 115-20, 122-3.
29. Morris G.P., Beck P.L., Herridge M.S., Depew W.T., Szewczuk M.R. and Wallace J.L.: Hapten-induced model of chronic inflammation and ulceration in the rat colon. Gastroenterol. 1989; 96: 795-803.
30. Dengiz G.O. and Gursan N.: Effects of Momordica charantia L. (Cucurbitaceae) on indomethacin-induced ulcer model in rats. Turk. J. Gastroenterol. 2005; 16(2): 85-88.
31. Krawisz J.E., Sharon P. and Stenson W.F.: Quantitative assay for acute intestinal inflammation based on myeloperoxidase activity. Gasteroenterol. 1984; 87: 1344-50.
32. Miranda K., Espy M.G. and Wink D.A.: A rapid and simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric Oxide 2001; 5: 62-71.
33. Elson C.O., Sartor R.B., Tennyson G.S. and Riddell R.H.: Experimental models of inflammatory bowel disease. Gastroenterol. 1995; 109: 1344-67.
34. Sakat S.S., Juvekar A.R. and Gambhire M.N.: Invitro antioxidant and anti-inflammatory activity of methanol extract of *Oxalis corniculata* Linn. Int. J. Pharm. Pharm. Sci. 2010; 2(1): 146-155.
35. Sharon P. and Stenson W.F.: Metabolism of arachidonic acid in acetic acid colitis in rats: similarity to human inflammatory bowel disease. Gastroenterol. 1985; 88: 55-63.
36. MacPherson B. and Pfeiffer C.: Experimental production of diffuse colitis in rats. Digestion 1978; 17: 135-50.
37. Rachmilewitz D., Simon P.L., Schwartz L.W., Griswald D.E., Fondacaro J.D. and Wasserman M.A.: Inflammatory mediators of experimental colitis in rats. Gastroenterol. 1989; 97: 326-7.
38. Cho E.J., Shin J.S., Noh Y.S., Cho Y.W., Hong S.Y., Park J.H. and *et al.*: Anti-inflammatory effects of methanol extract of *Patrinia scabiosaefolia* in mice with ulcerative colitis. J. Ethnopharmacol. 2010; 78: 120-27.
39. Baker S. and Campbell L.: Enterocyte injury by oxygen-dependent processes. Gastroenterol. 1991; 101: 716-20.
40. Kettle A.J., Gedye C.A. and Winterbourn C.C.: Mechanism of inactivation of myeloperoxidase by 4-aminobenzoic acid hydrochloride. Biochem. J. 1997; 321: 503-8.
41. Gow A.J., Farkouh C.R., Munson D.A., Posencheg M.A. and Ischiropoulos H.: Biological significance of nitric oxide-mediated protein modifications. American J. Physiol. Lung Cell Mol. Physiol. 2004; 287: L262-8.
42. Yano H., Hirayama F., Arima H. and Uekama K.: Prednisolone-appended cyclodextrin: alleviation of systemic adverse effect of prednisolone after intracolonic administration in 2, 4, 6-trinitrobenzenesulfonic acid-induced colitis rats. J. Pharma. Sci. 2001; 90: 2103.
43. Ross I.A.: Medicinal plant of the world. Humana press Inc; New Jercy: 1999. p. 197-221.
44. Chatterjee A. and Chandra P.S.: The Treatise on Indian medicinal plants. Vol 4th. New Delhi; National institute of science communication and information resources. 1993. p. 39-41.
45. Straub O.: Key to Carotenoids. In: Pfander F, editor. 2nd ed. Birkhauser Verlag; Basel, 1987. p. 296.
46. Olson J.A.: Provitamin A function of carotenoids: the conversion of beta-carotene into vitamin A. J. Nutr. 1989; 119: 105-8.