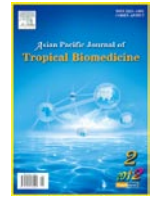




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Effect of aqueous extract of *Cucumis sativus* Linn. fruit in ulcerative colitis in laboratory animals

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ABSTRACT

Objective: To elucidate the ameliorative effect of aqueous extract of fruit of *Cucumis sativus* (*C. sativus*) (CS) in acetic acid induced colitis in wistar rats. **Methods:** The animals were administered with 2 mL acetic acid (4%) via intra rectal. The animals were divided into various treatment groups ($n=6$). Prednisolone was used as standard drug and *C. sativus* was administered at a dose of 100, 250 and 500 mg/kg p.o. The control group of animals received 1 mL of vehicle (distilled water). Ulcer area, ulcer index, spleen weight, colon weight to length ratio, macroscopic score, hematological parameters, colonic myeloperoxidase (MPO) and histological changes were recorded after the treatment regimen of 11 d. **Results:** Intrarectal instillation of acetic acid caused enhanced ulcer area, ulcer index, spleen weight, colon weight to length ratio, colonic MPO and hematological parameters. Pretreatment with *C. sativus* for 7 d exhibited significant effect in lowering of ulcer area, ulcer index as well as neutrophil infiltration at a dose of 250 and 500 mg/kg in acetic acid induced colitis. **Conclusion:** The present investigation demonstrates *C. sativus* is of potent therapeutic value in the amelioration of experimental colitis in laboratory animals by inhibiting the inflammatory mediator.

1. Introduction

The term inflammatory bowel disease includes ulcerative colitis and Crohn's disease. Ulcerative colitis is a chronic, recurrent disease characterized by diffuse mucosal inflammation of colon[1,2]. Ulcerative colitis invariably involves rectum and may extend proximally in continues fashion to involve part or all of colon.

Although etiology of inflammatory bowel disease (IBD) is unknown it appears that an abnormal response of the mucosal innate immune system to luminal bacteria may trigger inflammation which is perpetual by dysregulation of cellular immunity[3–5]. Crohn's and ulcerative colitis may be associated in 50% patient with number of extra intestinal manifestation including oral ulcers, oligo-articular or polyarticular non-deforming peripheral arthritis, spondylitis, sacroillitis, erythema, hepatitis, sclerosis[6,7]. Genetic modulation, infective agents, immunological disturbance, smoking, microorganism have been implicated

to orchestrate mucosal inflammation, hemorrhage and development of strictures in the colon[8,9].

Acetic acid induced colitis exhibit increased oxidation and lipid peroxidation during initiation of colitis. It also associated with alterations in the mucosal antioxidant defenses in UC. It is well accepted that ROS play an important role in the pathogenesis of IBD. Increased ROS production resulting from respiratory burst of infiltrating phagocytic cells which causes and decreased antioxidant capacity is a major pathogenic mechanism in IBD[10].

Despite of massive research there are no specific therapies have been developed for this diseases[11,12]. A number of biological therapies are available in clinical testing that more narrowly target various components of immune system[13]. Biological agents are highly effective for patients with corticosteroids dependence or refractory disease and potentially may improve the natural history of disease. The chief lines of therapy are 5-aminosalicylic acid derivatives, sulfasalpyridine (SASP), cortico-steroids, immune modulating agent etc. But their adverse reactions during prolonged treatment and the high relapse rate limit their use[14]. Hence there is severe need to develop chief lines medication to treat IBD.

Herbal drugs provide a faint ray of hope. Polyphenols, flavonoids have been shown to alleviate chronic inflammation in experimental model of IBD[15].

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Cucumis sativus (*C. sativus*) have been evaluated for a wide spectrum of activity including diuretic, Antihyperglycemic, Antioxidant, Amyolytic, Anticancer and analgesic activities using various *in-vitro* and *in-vivo* models^[16–22]. Hence, the objective of present investigation was to evaluate the ameliorative effect of *C. sativus* in acetic acid induced colitis model of IBD in laboratory animals.

2. Material and methods

2.1. Collection of plant material

The fruits of *C. sativus* L. were collected from rural areas of Pune district, Maharashtra in the month of December 2010. Authentication of Plant was carried out by P.G. Diwakar, Joint Director, Botanical Survey of India, Pune. The voucher specimen number was MIPCUSA1.

2.2. Preparation of extract

The plant material cut into small pieces and was macerated 6 L of water for about 48 h with occasional shaking. Macerate was decanted and filtered through cloth and then through filter paper to obtain a clear extract. Macerates were pooled and collected in trays and evaporated to dryness at 30–35 °C to obtain dried extract. The yield was found to be equal to 55 g (5.5%). Solution of *C. sativus* aqueous extract was prepared by using distilled water as vehicle^[21].

2.3. Preliminary phytochemical screening

The Preliminary phytochemical screening of the above extract of *C. sativus* L. was carried out according to the method described by Khandelwal *et al*^[23] and Patil *et al*^[24]. Phytochemicals analysis of the extract was performed for the identification of phytochemicals like alkaloid, flavonoids, steroid & phenols etc.

2.4. Animals

Healthy adult male swiss albino mice (20–30 g) and male wistar rats (230–250 g) were obtained from the National Institute of Biological sciences, Pune (India). The animals were housed in groups of 6 in solid bottom polypropylene cages. They were maintained at (24 ± 1) °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 (Pranav Agro industries Ltd., Sangli, India) throughout the experimental protocol, with the exception of overnight fasting before induction of the ulcer. The animals were provided with filtered water. The pharmacology and acute toxicity protocols were approved by the Institutional Animal Ethics Committee (IAEC) (522/05/ac/CPCSEA).

2.5. Drugs and chemicals

Acetic acid, anaesthetic ether, ethanol, formalin, hexadecyltrimethylammonium bromide, O–dianisidine dihydrochloride, hydrogen peroxide, disodium hydrogen

orthophosphate, potassium dihydrogen orthophosphate, carbon tetrachloride, chloroform, ether, hydrochloric acid and conc. Sulphuric acid were purchased from S.D. Fine Chemicals, Mumbai, India.

2.6. Acute toxicity testing

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

2.7. Dosages of *C. sativus* extract and standard drugs used

The Freshly prepared aqueous solution of *C. sativus* in the three different dosages (100 mg/kg, 250 mg/kg and 500 mg/kg) was administered to animals orally for 7 d. On 8th day, the disease was induced by acetic acid. The drug treatment was continued even after administration of acetic acid. Standard drug used for comparison was prednisolone. Prednisolone was not given as pretreatment. It was given on the day of acetic acid administration. Prednisolone was given in a dose of 2 mg/kg/day orally in rats as suspension containing 0.5% of sodium CMC^[2].

2.8. Induction of colitis

Colonic inflammation was induced in fasted rats following the method of Mascolo *et al*^[25]. The study comprised six different groups of six animals each as follows:

Group I – Normal animals: received 1 mL of distilled water for 11 d.

Group II – Control animals: received 2 mL of 4% acetic acid solution (once, intrarectally) and 1 mL of distilled water for 11 d.

Group III – Drug treated animals: received 7 d pretreatment with *C. sativus* (100 mg/kg p.o.) and 2 mL of 4% acetic acid solution, intrarectally on 8th day. Drug treatment was continued till 11th day.

Group IV – Drug treated animals: received 7 d pretreatment with *C. sativus* (250 mg/kg p.o.) and 2 mL of 4% acetic acid solution, intrarectally on 8th day. Drug treatment was continued till 11th day.

Group V – Drug treated animals: received 7 d pretreatment with *C. sativus* (500 mg/kg 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 animals: received Prednisolone (2 mg/kg, p.o., for 3 d) and acetic acid (2 mL of 4% solution, once, intrarectally). Prednisolone and acetic acid treatment was started on the same day.

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

2.9. Evaluation of the disease

The disease induced in experimental animals was evaluated based on its macroscopic characteristics. Evaluation pattern for macroscopic characteristics, reported by Morris *et al*[26] was used after some modifications.

2.9.1. Determination of ulcer area and ulcer index

The Evaluation of Ulcer area and Ulcer index was performed according to Dengiz *et al*[27] and Kandhare *et al*[28]. For determination of ulcer area, each colon was incised and washed with normal saline and image was captured. The images were processed using image J software and adobe Photoshop to determine ulcer area.

2.9.2. Determination of hematological parameters

Hematological parameters were determined using an automated haematological analyzer (Sysmex KX–21) with specific software for rat blood samples[29]. The parameters analyzed were white blood cell (WBC) number, red blood cell (RBC) number, haemoglobin (Hb) concentration, hematocrit (HCT) and platelet count (PLT).

2.9.3. Biochemical assays

Samples from the colon were stored immediately at -80°C till analysis. Tissue samples were homogenized in 10 mmol Tris–HCl buffer (pH 7.1) and the homogenate was used for the measurement of Myeloperoxidase (MPO) in colon tissue.

2.9.3.1. 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*[30] and Raygude *et al*[31]. Myeloperoxidase activity was defined as the quantity of enzyme degrading $1\ \mu\text{mol}$ of peroxide per min at 25°C and was expressed in units per gram (U/g) of wet scrapings.

2.9.4. Evaluation based on microscopic (histologic) characters

Freshly excised colon of one animal from each group was washed with saline and preserved in 10% formaldehyde solution for histopathological studies. It processed for 12 h using isopropyl alcohol, xylene and paraffin embedded for light microscopic study. Paraffin embedded tissue section cut at $5\ \mu\text{m}$ thickness were prepared and stained after deparaffination using hematoxyline and eosin stain to verify morphological assessment of colon damage. Photomicrographs were captured at a magnification of $40\times$.

2.10. Data and statistical analysis

All the results were expressed as mean \pm S.E.M. Data analysis was performed using GraphPad Prism 5.0 software (GraphPad, San Diego, CA). 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 tests was applied for post hoc analysis. A value of $P<0.05$ was considered to be statistically significant.

3. Results

3.1. Acute toxicity testing

Acute toxicity studies of the aqueous extract of *C. sativus* shows no signs and symptoms such as restlessness, respiratory distress, diarrhea, convulsions and coma and it was found safe up to $5000\ \text{mg/kg}$.

3.2. Preliminary phytochemical screening

The aqueous extract of *C. sativus* L. fruit was screened for various chemical tests as per the reported methods and was found to contain Alkaloids, tannin, steroids, flavanoids, cardiac glycosides and carbohydrate.

3.3. Acetic acid-induced colitis

Intrarectal instillation of acetic acid (4%) caused inflammatory reaction in the colon. The inflammation occurred in rectum and distal colon portion. *C. sativus* treated group showed mild lesions, regeneration and inflammatory reaction. The prednisolone treated group showed suppressed inflammatory reaction.

3.3.1. Effect of *C. sativus* on colon weight

In the Acetic acid control animals colon weight (2.62 ± 0.09) g was increased significantly ($P<0.001$) as compared to Normal group (1.12 ± 0.17) g. Pretreatment of *C. sativus* ($500\ \text{mg/kg}$, p.o.) for 7 d decrease the colon weight (1.40 ± 0.13) g significantly ($P<0.001$) as compared to acetic acid control group (Table 1).

3.3.2. Effect of *C. sativus* colon width

The mean colon width of acetic acid control animals was found to be increased (1.55 ± 0.10) mm significantly ($P<0.001$) as compared to normal group (0.55 ± 0.04) mm. Pretreatment of *C. sativus* ($500\ \text{mg/kg}$, p.o.) for 7 d decreased the colon width (0.81 ± 0.08) mm significantly ($P<0.001$) as compared to

Table 1.

Effect of *C. sativus* on colon weight, colon width, colon weight to length ratio, spleen weight and macroscopic score of rat in acetic acid induced IBD.

Group	Colon weight (g)	Colon width (cm)	Colon weight to length ratio	Spleen weight (g)	Macroscopic score
Normal	1.12 ± 0.17	0.55 ± 0.04	0.141 ± 0.022	1.13 ± 0.10	0.00 ± 0.0
Acetic acid control	2.62 ± 0.09	1.55 ± 0.10	0.300 ± 0.022	2.07 ± 0.06	9.66 ± 0.33
Prednisolone (2 mg/kg)	$1.27 \pm 0.13^{***}$	$0.66 \pm 0.06^{***}$	$0.134 \pm 0.014^{***}$	$1.28 \pm 0.15^{**}$	$2.80 \pm 0.37^{***}$
<i>C. sativus</i>	100 mg/kg	2.30 ± 0.10	1.45 ± 0.07	1.87 ± 0.06	8.66 ± 0.33
	250 mg/kg	2.08 ± 0.06	1.25 ± 0.09	1.65 ± 0.10	$7.00 \pm 0.57^{**}$
	500 mg/kg	$1.40 \pm 0.13^{***}$	$0.81 \pm 0.08^{***}$	$1.36 \pm 0.08^{**}$	$4.25 \pm 0.62^{***}$

Data are expressed as mean \pm 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 2.Effect of *C. sativus* on ulcer area and ulcer index of rat in acetic acid induced IBD.

Parameter	Normal	Acetic acid control	Prednisolone (2 mg/kg)	<i>C. sativus</i>		
				100 mg/kg	250 mg/kg	500 mg/kg
Ulcer area (mm ²)	0.00 ± 0.00	30.00 ± 1.15	6.40 ± 1.20***	23.00 ± 2.08**	23.00 ± 2.08**	12.25 ± 0.85***
Ulcer index	0.00 ± 0.00	58.05 ± 1.59	13.21 ± 2.24***	42.20 ± 5.37**	50.91 ± 2.20*	26.34 ± 1.50***

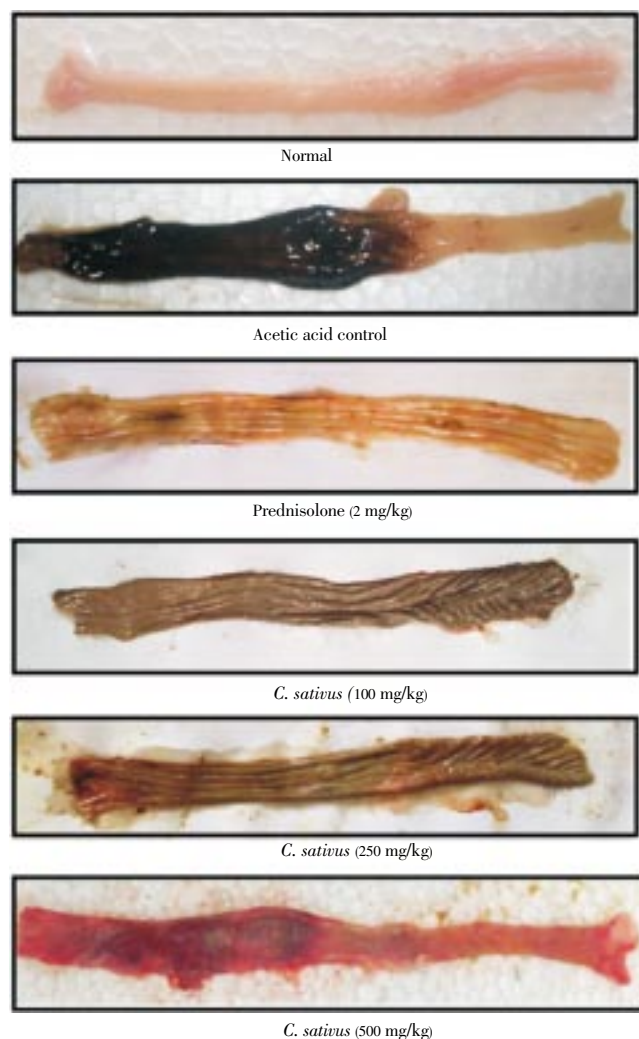
Data are expressed as mean ± S.E.M. from five rats and analyze by One Way ANOVA followed by Dunnett's test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ as compared to acetic acid control group.

Table 3.Effect of *C. sativus* on hematological parameter of rat in acetic acid induced IBD.

Parameter	Normal	Acetic acid control	Prednisolone (2 mg/kg)	<i>C. sativus</i>		
				100 mg/kg	250 mg/kg	500 mg/kg
RBC ($\times 10^6 / \mu\text{L}$)	9.70 ± 0.26	6.94 ± 0.18	9.05 ± 0.12**	7.02 ± 0.57	7.57 ± 0.02	8.88 ± 0.00**
HGB (g/dL)	14.63 ± 0.29	12.90 ± 0.10	15.30 ± 0.05**	14.49 ± 0.69*	14.35 ± 0.35*	14.50 ± 0.50*

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.

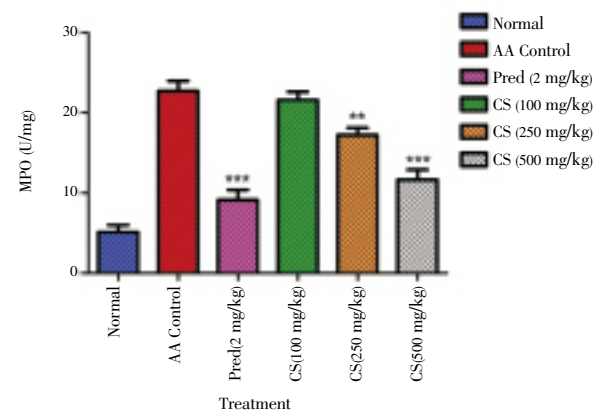
acetic acid control group (Table 1).

**Figure 1.** Morphological representation of colon treated with acetic acid, Prednisolone and *C. sativus*.

3.3.3. Effect of *C. sativus* on colon weight to length ratio

The ratio of colon weight/length was found to be increased (0.300 ± 0.022) significantly ($P < 0.001$) in acetic acid control group as compared to normal group (0.1411 ± 0.022). Pretreatment of *C. sativus* (500 mg/kg, p.o.), for 7 d decreased the colon weight/length ratio (0.155 ± 0.017) was found to be

significant ($P < 0.01$) as compared to acetic acid control group (Table 1).

**Figure 2.** Effect of *C. sativus* on colonic MPO concentrations of rats 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.

3.3.4. Effect of *C. sativus* on spleen weight

In the present study, the rat with acetic acid induced colitis exhibited splenic enlargement (2.07 ± 0.06) g as compared with Normal (1.13 ± 0.10) g, ($P < 0.001$). Pretreatment of *C. sativus* (500 mg/kg, p.o.) for 7 d inhibiting splenic enlargement (1.36 ± 0.08) g was found to be significant ($P < 0.01$) as compared to acetic acid control group (Table 1).

3.3.5. Effect of *C. sativus* macroscopic scores

The colons of the rats were examined macroscopically for signs of hemorrhage and ulceration by an independent observer, in a blinded fashion, using a previously established scoring system 21. The mean macroscopic score of acetic acid control group (9.66 ± 0.33) was found to be significantly ($P < 0.001$) increased as compared to normal group. Pretreatment of *C. sativus* (250 mg/kg, 500 mg/kg, p.o.) had decreased macroscopic score of colon (7.00 ± 0.57 and 4.25 ± 0.62 resp.) significantly ($P < 0.01$ and $P < 0.001$ resp.) as compared to acetic acid control group and reduce the inflammation and ulceration of colon. (Figure 1 & Table 1)

3.3.6. Effect of *C. sativus* on ulcer area

Rectal administration of 6% acetic acid produced ulcers of colon in acetic acid control and all drug treated animals. The mean ulcer area of acetic acid control group was $(30.00 \pm 1.15) \text{ mm}^2$ showed high ulcerogenic effect of acetic acid. Pretreatment of *C. sativus* (100, 250, 500 mg/kg, p.o.) for 7 d decreased the ulcer area of colon ($23.00 \pm 2.08 \text{ mm}^2$, $(23.00 \pm 2.08) \text{ mm}^2$, $(12.25 \pm 0.85) \text{ mm}^2$ resp. significantly ($P < 0.05$, $P < 0.01$ and $P < 0.001$ resp.) (Table 2).

3.3.7. Effect of *C. sativus* on ulcer index

The mean ulcer index of acetic acid control group was (58.05 ± 1.59) showed high ulcerogenic effect of acetic acid. Pretreatment of *C. sativus* (500 mg/kg, p.o.) for 7 d decreased the ulcer index of colon (26.34 ± 1.50) significantly ($P < 0.001$). *C. sativus* (100 mg/kg, p.o.) was more effective in reducing ulcer index of colon than *C. sativus* (250 mg/kg, p.o.) (Table 2).

3.3.8. Effect of *C. sativus* on hematology

Table 3 summarizes the Effect of *C. sativus* on hematological parameter of acetic acid control and experimental animals. The acetic acid treated rats showed a significant decrease in the hematological parameters RBC count, Hb compared to normal rats (9.70 ± 0.26 and 14.63 ± 0.29 resp.). These reductions were significantly attenuated in the *C. sativus* pretreated group (8.88 ± 0.00 and 14.50 ± 0.50). No effect was observed in the rest of the hematological parameters analyzed.

3.3.9. Effect of *C. sativus* on colonic MPO concentrations

The colitis caused by acetic acid was associated with an increase in MPO activity ($22.74 \pm 1.26 \text{ U/mg}$). In the groups pre-treated with *C. sativus* (250 and 500 mg/kg, p.o.) for 7 d, the MPO activity of colonic mucosal scrapings were significantly decreased (17.26 ± 0.88 and $11.68 \pm 1.25 \text{ U/mg}$ resp.) ($P < 0.01$, $P < 0.001$), as compared with acetic acid control

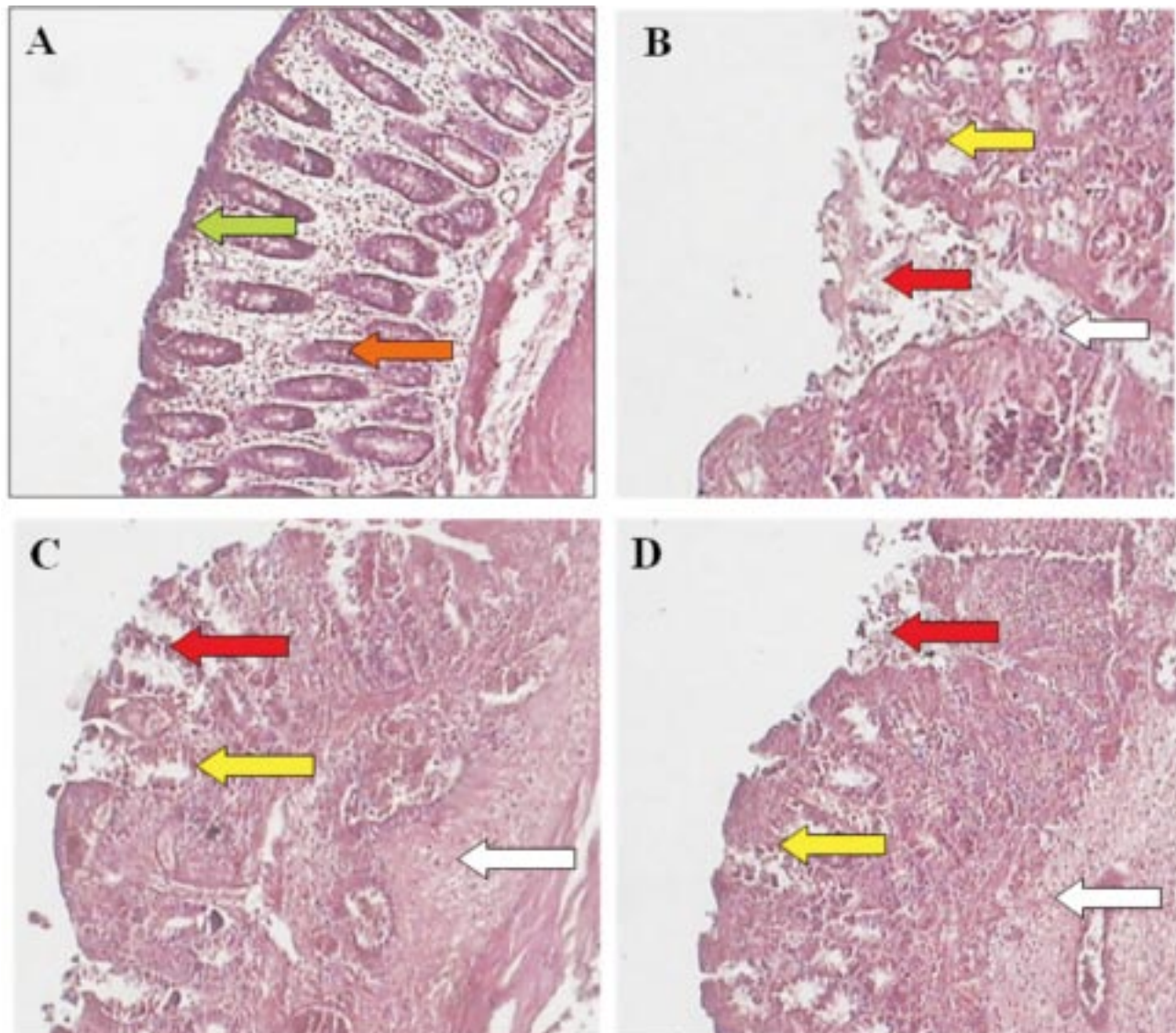


Figure 3. Photomicrographs of sections of colons from rats stained with H&E.

Colon microscopic image of (A) Normal rat with intact epithelial (orange arrow) and mucosal layer (green arrow); (B) Acetic acid induced colitis rat with extensive damage including edema in submucosa (red arrow) and cellular infiltration (white arrow), necrosis (yellow arrow) and ulceration; (C) Prednisolone (2 mg/kg, p.o.) treated rat with infiltration (white arrow), necrosis (yellow arrow) and hemorrhages (red arrow); (D) *C. sativus* (500 mg/kg p.o.) 7 d pretreated rat with edema in submucosa (white arrow), cellular infiltration (yellow arrow) and hemorrhages (red arrow). Images (40 magnification) are typical and representative of each study group.

group (Figure 2).

3.3.10. Histopathological results

We examined the H & E stained sections of ulcerated areas of the colons of the rats for signs of colitis. The histopathological features of untreated animals included transmural necrosis, edema and diffuse inflammatory cell infiltration in the mucosa, desquamated areas and loss of the epithelium, hyperemia and Goblet cell hyperplasia. An infiltrate consisted of mixed inflammatory cells was observed (Figure 3B). The polymorphonuclear neutrophil infiltration was found in affected tissues. Pretreatment of rats with *C. sativus* (500 mg/kg, p.o.) (Figure 3D) or treatment with prednisolone (Figure 3C) significantly attenuated the extent and severity of the histological signs of cell damage. In case of normal rats colon there were no inflammatory cells in the lamina propria and the epithelium remained intact (Figure 3A).

4. Discussion

Acetic acid induced colitis in laboratory rats has been used to screen various drugs with proposed disease modifying property[32–37]. Inflammatory bowel disease (IBD) is chronic relapsing conditions characterized by up-regulated pro inflammatory mediators and dysregulated immune responses resulting in tissue damage[38]. The genetics, immunology and environment are the multiple etiologic theories which are related with IBD.

In the present investigation, the aqueous extract of *C. sativus* L. selected for screening against experimentally induce inflammatory bowel disease. The intestinal mucosa in IBD is characterized by ongoing inflammation that results from activation of resident immune cells and infiltration of inflammatory cells from the circulation[39,40]. In the present investigation acetic acid was used to induce colonic lesions. The acetic acid causes massive localized erosion of the colonic mucosa leading to sever localized inflammation and haemorrhages.

Various parameters such as colonic weight, colonic length, colonic width, Colon weight/length ratio, macroscopic score, ulcer index, body weight, rectal bleeding, stool consistency and spleen weight were recorded in this animal model. Colonic weight, width and weight/length ratio is considered an index for local inflammation along with the other parameters of edema and wall thickening[41,42]. Colons were opened longitudinally and macroscopic damage score was given to each specimen. The results suggest that colon weight, width and weight/length ratio was found to be significantly increased in acetic acid control group as compared to normal group. The elevation in the colon weight and colon weight to length ratio was inhibited by *C. sativus* depicting its healing property.

Spleen is the indispensable part of immune system and reticuloendothelial system and has been found to be atrophied in chronic colitis[43]. The reduction in the splenic enlargement by the *C. sativus* provides an innuendo to the ability of *C. sativus* to modulate the immune system.

The gross morphological lesions characterised by ulcer and necrotic area of various sizes[44], were healed depicting protection of microflora from the corrosive effect of acetic acid by *C. sativus*. Ulcer area and ulcer index were

quantitatively determined and reflect the protective action of *C. sativus*.

Clinical manifestation of IBD includes exacerbated hematological imbalance leading to unexplained diarrhoea and Malena. This feature was reversed in *C. sativus* treated animals showing its therapeutic potential. Hematology provides an insight into the disease state of colitis. The various components of blood were disproportionately altered[45–47]. In the vehicle treated group where as their ratios were unchanged in *C. sativus* treated animals.

Intrarectal administration of acetic acid leads to protonation and migration of acetic acid molecule in to colonic microflora after internalization to produce protons leading to epithelial denudation[48]. This process instigates infiltration of neutrophils into tissue. The surge of neutrophil infiltration into tissue is a direct evidence of pathogenicity. The level of MPO is a measure a degree of neutrophil infiltration[49–55]. The free radical contributes to initiate and propagate a vicious cycle. *C. sativus* restores the elevated level of MPO.

Alkaloids, steroids, flavonoids, polyphenols have been proven antioxidant, anti-inflammatory and immunomodulatory active principles[56,57]. These phytoconstituents of *C. sativus* may have synergistically contributed to the attenuation of ulcerative colitis.

From the results it could be deduced that *C. sativus* possesses potent activity against various pathological changes caused by administration of acetic acid. *C. sativus* was found to be effective in both post treatment and pretreatment given in acetic acid models. It could be hypothesized that the drug may form a layer over the colonic mucosa and also reduce localize inflammatory processes. The drug possesses free radical scavenging profile[16,17]. Hence, it was able to reverse the localized inflammatory response and heal the hemorrhagic lesions which were caused by the administration of acetic acid. The results provide an idea to the probable mechanism of action of the drug. However, further studies need to be carried out to enables to zero upon a full proof mechanism of action of *C. sativus* L.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

- [1] Baumgart DC, Carding SR. Inflammatory bowel disease: cause and immunobiology. *Lancet* 2007; **369**(9573): 1627–1640.
- [2] Patil MVK, Kandhare AD, Bhise SD. Anti-inflammatory effect of *Daucus carota* root on experimental colitis in rats. *Int J Pharm Pharm Sci* 2012; **4**(1): 337–343.
- [3] Ghosh P, Kandhare AD, Gauba D, Raygude KS, Bodhankar SL. Determination of efficacy, adverse drug reactions and cost effectiveness of three triple drug regimens for the treatment of *H. pylori* infected acid peptic disease patients. *Asian Pac J Trop Biomed* 2012. In press.
- [4] Ghosh P, Kandhare AD, Raygude KS, Gauba D, Gosavi TP, Bodhankar SL. Cigarette smoking and *H. pylori* infection: A meta-analysis of literature. *Der Pharmacia Lettre* 2012; **4**(1): 128–134.
- [5] Raygude KS, Kandhare AD, Ghosh P, Gosavi TP, Bodhankar SL. Consumption of Alcohol and *H. pylori* infection: A Cumulative Meta-Analysis of Literature. *Asian J Biochem Pharm Res* 2011; **3**(1): 338–345.
- [6] Gosavi TP, Ghosh P, Kandhare AD, Bodhankar SL. Therapeutic effect of *H. pylori* nosode in healing of chronic *H. pylori* infected ulcers in laboratory animals. *Asian Pac J Trop Dis* 2012. In press.
- [7] Kandhare AD, Raygude KS, Ghosh P, Gosavi TP, Bodhankar SL. A comparative cross sectional descriptive study of individual pharmacy Vs mall pharmacies to evaluate customer satisfaction as well as purchasing behavior. *Der Pharmacia Lettre* 2011; **3**(4): 71–83.
- [8] Strober W, Fuss IJ. Proinflammatory cytokines in the pathogenesis of inflammatory bowel diseases. *Gastroenterol* 2011; **140**(6): 1756–1767.
- [9] Kozuch PL, Hanauer SB. Treatment of inflammatory bowel disease: A review of medical therapy. *World J Gastroenterol* 2008; **14**(3): 354–377.
- [10] Mohsen M, Alireza G, Parvin M, Elham JS. Comparative study of *Berberis vulgaris* fruit extract and Berberine chloride effects on acetic acid induced colitis in rats. *Iranian J Pharm Res* 2011; **10**(1): 97–104.
- [11] Patil MVK, Kandhare AD, Bhise SD, Bhale S. A comparative cross sectional descriptive market survey of various brands of Levofloxacin. *Asian J Pharm Biol Res* 2011; **1**(2): 174–181.
- [12] Patil MVK, Kandhare AD, Bhise SD. Evaluation of patient information leaflets on basis of consumer psychology and opinion. *Int J Pharm Pharm Sci Res* 2011; **1**(2): 87–92.
- [13] Gosavi TP, Ghosh P, Kandhare AD, Bodhankar SL. Unwrapping Homeopathic Principles in the Wake of Research: Serendipity, Placebo or True Therapeutic Milestones. *Pharmacologyonline* 2011; **1**: 894–906.
- [14] Joshi R, Kumar S, Unnikrishnan M, Mukherjee T. Free radical scavenging reactions of sulfasalazine, 5-aminosalicylic acid and sulfapyridine: mechanistic aspects and antioxidant activity. *Free Radic Res* 2005; **39**: 1163–1172.
- [15] Rahimi R, Mozaffari S, Abdollahi M. On the use of herbal medicines in management of inflammatory bowel diseases: a systematic review of animal and human studies. *Dig Dis Sci* 2009; **54**: 471–480.
- [16] Gill NS, Bajwa J, Sharma P, Dhiman K, Sood S, Sharma PD, et al. Evaluation of antioxidant and antiulcer activity of traditionally consumed *Cucumis melo* seeds. *J Pharmacol Toxicol* 2011; **6**: 82–89.
- [17] Chu YF, Sun J, Wu X, Liu RH. Antioxidant and antiproliferative activities of common vegetables. *J Agric Food Chem* 2002; **50**(23): 6910–6916.
- [18] Pellegrini N, Serafini M, Colombi B, Del Rio D, Salvatore S, Bianchi M, et al., Total antioxidant capacity of plant foods, beverages and oils consumed in Italy assessed by three different in vitro assays. *J Nutr* 2003; **133**: 2812–2819.
- [19] Repka V, Fischerova I. Induction and distribution of amyolytic activity in *Cucumis sativus* L. in response to virus infection. *Acta Virologica* 1999; **43**(4): 227–235.
- [20] Villasenor IM, Simon MK, Villanueva AM. Comparative potencies of nutraceuticals in chemically induced skin tumor prevention. *Nutr Cancer* 2002; **44**(1): 66–70.
- [21] Patil MK, Kandhare AD, Bhise SD. Pharmacological evaluation of ameliorative effect of aqueous extract of *Cucumis sativus* L. fruit formulation on wound healing in Wistar rats. *Chron Young Sci* 2011; **2**: 207–213.
- [22] Nadkarni AK, Nadkarni KM. *Indian Materia Medica*. Bombay: Popular Prakashan; 2005, p. 403–404.
- [23] Khandelwal KR. *Practical pharmacognosy, technique and experiments*. 8th ed. Pune: Nirali Prakashan; 2007, p. 149–153.
- [24] Patil MVK, Kandhare AD, Bhise SD. Anti-arthritis and anti-inflammatory activity of *Xanthium strumarium* L. ethanolic extract in Freund's complete adjuvant induced arthritis. *Biomed Aging Pathol* 2012; **2**: 6–15.
- [25] Mascolo N, Izzo A, Autore G, Maiello F, Di Carlo G, Capasso F. Acetic acid-induced colitis in normal and essential fatty acid deficient rats. *J Pharmacol Exp Ther* 1995; **272**: 469–475.
- [26] Morris GP, Beck PL, Herridge MS, Depew WT, Szewczuk MR, Wallace JL. Hapten-induced model of chronic inflammation and ulceration in the rat colon. *Gastroenterol* 1989; **96**: 795–803.
- [27] Dengiz GO, Gursan N. Effects of *Momordica charantia* L. (Cucurbitaceae) on indomethacin-induced ulcer model in rats. *Turkish J Gastroenterol* 2005; **16**(2): 85–88.
- [28] Kandhare AD, Raygude KS, Ghosh P, Bodhankar SL. The ameliorative effect of fisetin, a bioflavonoid, on ethanol-induced and pylorus ligation-induced gastric ulcer in rats. *Int J Green Pharm* 2011; **5**: 236–243.
- [29] Larrosa M, González-Sarrías A, Yáñez-Gascón MJ, Selma MV, Azorín-Ortuño M, Toti S, et al. Anti-inflammatory properties of a pomegranate extract and its metabolite urolithin-A in a colitis rat model and the effect of colon inflammation on phenolic metabolism. *J Nutr Biochem* 2009; **21**(8): 717–725.
- [30] Krawisz JE, Sharon P, Stenson WF. Quantitative assay for acute intestinal inflammation based on myeloperoxidase activity. *Gastroenterol* 1984; **87**: 1344–1350.
- [31] Raygude KS, Kandhare AD, Ghosh P, Ghule AE, Bodhankar SL. Evaluation of ameliorative effect of quercetin in experimental model of alcoholic neuropathy in rats. *Inflammopharmacol* 2012 DOI 10.1007/s10787-012-0122-z.
- [32] Channel DV, Bariya AH, Deshpande SS. Natural agent for inflammatory bowel disease. *Int J Res Ayu Pharm* 2011; **2**(1): 84–89.
- [33] Moussata D, Goetz M, Gloeckner A, Kerner M, Campbell B, Hoffman A, et al. Confocal laser endomicroscopy is a new imaging modality for recognition of intramucosal bacteria in inflammatory bowel disease in vivo. *Gut* 2011; **60**: 26–33.

- [34] Kandhare AD, Raygude KS, Ghosh P, Gosavi TP, Bodhankar SL. Patentability of Animal Models: India and the Globe. *Int J Pharm Biol Arc* 2011; **2**(4): 1024–1032.
- [35] Isaacs K, Herfarth H. Role of probiotic therapy in IBD. *Inflamm Bowel Dis* 2008; **14**: 1597–1605.
- [36] Su L, Shen L, Clayburgh DR, Nalle SC, Sullivan EA, Meddings JB, et al. Targeted epithelial tight junction dysfunction cause immune activation and contributes to development of experimental colitis. *Gastroenterol* 2009; **136**: 551–563.
- [37] UK IBD Genetics Consortium, Barrett JC, Lee JC, Lees CW, Prescott NJ, Anderson CA, et al; Genome-wide association study of ulcerative colitis identifies three new susceptibility loci, including the HNF4A region. *Nat Genet* 2009; **41**: 1330–1334.
- [38] Munoz FS, Lopez AD, Furusho JKY. Role of cytokines in inflammatory bowel disease. *World J Gastroenterol* 2008; **14**(27): 4280–4288.
- [39] Thippeswamy BS, Mahendran S, Biradar MI, Raj P, Srivastava K, Badami S, et al. Protective effect of embelin against acetic acid induced ulcerative colitis in rats. *Eur J Pharmacol* 2011; **654**(1): 100–105.
- [40] Darji VC, Bariya AH, Deshpande SS, Patel DA. Effect of Punica granatum fruit in inflammatory bowel disease. *J Pharm Res* 2010; **3**(12): 2850–2852.
- [41] Wu LH, Xu ZL, Dong D, He SA, Hong Y. Protective effect of Anthocyanins extract from blubbery on TNBS-induced IBD of mice. *Evid Based Complement Alternat Med* 2011; **2011**: 525462.
- [42] Deshmukh CD, Veeresh B, Pawar AT. Protective effect of Emblica officinalis fruit extract on acetic acid induced colitis in rats. *J Herbal Med Toxicol* 2010; **4**(2): 83–87.
- [43] Cho EJ, Shin JS, Noh YS, Cho YW, Hong SY, Park JH, et al. Anti-inflammatory effects of methanol extract of *Patrinia scabiosaefolia* in mice with ulcerative colitis. *J Ethnopharmacol* 2010; **78**: 120–127.
- [44] Gandhi TR, Darji VC, Solanki RP, Patel KV. Effect of *Aegle marmelos* Linn. (Rutaceae) fruit in inflammatory bowel disease. *Indian Drugs* 2009; **46**(6): 460–464.
- [45] Jagtap AG, Niphadkar PV, Phadke AS. Protective effect of aqueous extract of *Bombax malabaricum* DC on experimental models of inflammatory bowel disease in rats and mice. *Indian J Exp Biol* 2011; **49**(5): 343–351.
- [46] Kandhare AD, Raygude KS, Ghosh P, Ghule AE, Bodhankar SL. Neuroprotective effect of naringin by modulation of endogenous biomarkers in streptozotocin induced painful diabetic neuropathy. *Fitoterapia* 2012; **83**: 650–659.
- [47] Kandhare AD, Raygude KS, Ghosh P, Ghule AE, Bodhankar SL. Therapeutic role of curcumin in prevention of biochemical and behavioral aberration induced by alcoholic neuropathy in laboratory animals. *Neurosci Lett* 2012; **511**: 18–22.
- [48] Abdolghaffari AH, Baghaei A, Moayer F, Esmaily H, Baeeri M, Monsef-Esfahani HR, et al. On the benefit of *Teucrium* in murine colitis through improvement of toxic inflammatory mediators. *Hum Exp Toxicol* 2010; **29**: 287–295.
- [49] Hagar HH, Medany AE, Eter EE, Arafa M. Ameliorative effect of pyrrolidinedithiocarbamate on acetic acid-induced colitis in rats. *Eur J Pharmacol* 2007; **554**: 69–77.
- [50] Pawar P, Gilda S, Sharma S, Jagtap S, Paradkar A, Mahadik K, et al. Rectal gel application of *Withania somnifera* root extract expounds anti-inflammatory and mucorestorative activity in TNBS-induced inflammatory bowel disease. *BMC Compl Alt Med* 2011; **11**: 34–42.
- [51] Gosavi TP, Kandhare AD, Ghosh P, Bodhankar SL. Anticonvulsant activity of *Argentum metallicum*, a homeopathic preparation. *Der Pharmacia Lettre* 2012; **4**(2): 626–637.
- [52] Patil MVK, Kandhare AD, Ghosh P, Bhise SD. Determination of role of GABA and nitric oxide in anticonvulsant activity of *Fragaria vesca* L. ethanolic extract in chemically induced epilepsy in laboratory animals. *Orient Pharm Exp Med* 2012; In Press DOI 10.1007/s13596-012-0072-4.
- [53] Raygude KS, Kandhare AD, Ghosh P, Bodhankar SL. Anticonvulsant effect of fisetin by modulation of endogenous biomarkers. *Biomed Prev Nutr* 2012; In press <http://dx.doi.org/10.1016/j.bionut.2012.04.005>.
- [54] Amini-Shirazi N, Hoseini A, Ranjbar A, Mohammadirad A, Khoshakhlagh P, Yasa N, et al. Inhibition of tumor necrosis factor and nitrosative/oxidative stresses by *Ziziphora clinopoides* (Kahliti); a molecular mechanism of protection against dextran sodium sulfate-induced colitis in mice. *Toxicol Mech Methods* 2009; **19**: 183–189.
- [55] Kandhare AD, Raygude KS, Ghosh P, Ghule AE, Gosavi TP, Badole SL, Bodhankar SL. Effect of hydroalcoholic extract of *Hibiscus rosa sinensis* Linn. leaves in experimental colitis in rats. *Asian Pac J Trop Biomed* 2012; **2**(5): 337–344.
- [56] Patel MA, Patel PK, Patel MB. Aqueous extract of *Ficus bengalensis* Linn. bark for inflammatory bowel disease. *J Young Pharm* 2010; **2**(2): 130–136.
- [57] Patil MVK, Kandhare AD, Bhise SD. Pharmacological evaluation of ethanolic extract of *Daucus carota* Linn root formulated cream on wound healing using excision and incision wound model. *Asian Pac J Trop Biomed* 2012. In press.