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Effect of methanolic extract of *Cissampelos pareira* on Acetic acid and Dextran Sodium Sulphate (DSS) induced inflammatory bowel disease (IBD) in albino rats and mice

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Abstract

Effect of methanolic extract of *Cissampelos pareira* (CPME) (200, 400 mg/kg, PO) was studied on acetic acid and DSS induced colitis in rats and mice respectively. Montelukast (20 mg/kg, PO) was used as a Standard drug. At the end of experiment various parameters like scoring for colon, Disease activity index (DAI) which took into account body weight, stool consistency, gross bleeding, Alkaline phosphate (ALP), Erythrocyte Sedimentation Rate (ESR), white blood cell (WBC) count and myeloperoxydase (MPO) activity were measured. Treatment with CP (200,400 mg/kg, PO) decreased significantly macroscopic damage in both models. Histological analysis showed that both doses of the extract improved the microscopic structure and preserved some areas of the colonic mucosa structure. MPO, as a marker of neutrophils infiltration was decreased in a dose-dependent way. In addition DAI, WBC count, ESR and ALP activity also significantly decreases in both doses of CP ($p < 0.05$, $p < 0.01$ respectively) in both models. So, from our study we found that CP exerts marked protective effects in acute experimental colitis and may be used clinically in treatment of IBD. This protective effect may be due to the regulations of the productions and an expression of inflammatory mediators.

Keywords: *Cissampelos pareira*, inflammatory bowel disease, Acetic acid, DSS, Montelukast.

1. Introduction

Inflammatory bowel disease (IBD) is an immune-mediated disorder of gastrointestinal tract characterized by a chronic, relapsing and remitting inflammatory condition and ulceration [1]. The disease is clinically characterized by two overlapping phenotypes — ulcerative colitis (UC) and Crohn's disease (CD) [2]. UC is usually affected to the colon and rectum and consists of continuous involvement of variable severity ulceration, edema and hemorrhage along the length of the colon [3]. CD may affect any part of the GI tract from mouth to anus; causes skip lesions that can result in fistulas, perforations, or strictures. When only the colon is affected, CD can be difficult to distinguish from UC.

Even though the exact etiology of UC and CD is unknown, the causes of IBD involves absorption of different dietary supplements, combination of infection, genetic susceptibility, immunologic factors, microflora of gastrointestinal tract, smoking, environmental triggers and exposure to the chemicals. Apart from these, other synthetic drugs are also causes an IBD due to their inadequate use or adverse effect of drug used in other disease condition. The development of an abnormal immune response occurs, mainly by activated neutrophils, monocytes, macrophages and characterized by an enhanced formation of reactive oxygen and nitrogen species. Various proinflammatory cytokines such as interleukins (IL)-1, IL-6, interferon (INF)-γ, tumor necrosis factor alpha (TNF-α), Oxidant – mediated injury [4], chemolines and adhesion molecules are known to contribute to the pathogenesis of IBD.

Most of the current therapies for IBD involve treatment with glucocorticosteriods, immunomodulator such as azathioprine, 6-mercaptopurine and aminosalicylates (5-ASA), antibiotics. However all of these

drugs have short coming [5]. 5-ASA is well tolerated but diarrhea, cramps and abdominal pain are occasional side effects and this can be accompanied by a fever, rash or kidney problems. Corticosteroids have also been used for many years to treat patients and have well known side effects which include rounding of the face, acne, increase body hair, diabetes, weight gain and high blood pressure[5]. Immunosuppressive drugs have also been used to control severe illness, regardless of the more serious complications and toxic side effect associated with them[6]. Absorption of different dietary supplements, environment, microbial flora, genetic susceptibility and exposure to the chemicals, there is a chance of inflammation in bowel.

Apart from these, other synthetic drugs are also causing an inflammation in bowel due to their inadequate use or as adverse effect of drug used in other disease condition which is alien to the body organs.

Therefore it is necessary to search for an alternative drug in the treatment of inflammatory bowel disease and a need to develop safe and effective alternative therapeutic agents for treatment of inflammatory bowel disease.

Cissampelos pareira Linn. (CP) Hirsuta (family: Menispermaceae), commonly known as ambastha or laghupatha in Ayurveda, is a climbing shrub, with striate branches, juvenile or subglabrous distributed throughout tropical and subtropical part of India, warm parts of Asia, East Africa and America[7,8]. According to the uses mentioned in traditional medicine the root of plant is mainly used but the whole plant is used in asthma, dysentery, fever, heart trouble, itching, skin eruption, vomiting and a paste made from the root is used to treat fistula, pruritus, skin disorders and snake bites[9]. Antioxidant [10], anti- diarrheal [11], immunomodulatory [12], antimalarial [13], analgesic activity [14], anti-inflammatory activity [15], antifertility [16], antispasmodic [17], antinociceptive, anti arthritic activity [18] and diuretic activity [19], of the plant is scientifically reported.

To best of our knowledge no scientific data regarding the activity of CP on IBD is available in literature. Therefore, we have undertaken this research work to evaluate anti-IBD activity of CP by acetic acid induced colitis in rats and Dextran Sodium Sulphate (DSS) induced colitis in mice.

2. Material and methods:

2.1 Plant material:

Fresh plant of *Cissampelos pareira* Linn. was collected from the waste land near the farms in the month of November –December, 2011 and authenticated by Dr. Bimal S. Desai, Assistant Professor (Botanist), ASPEE College of Horticulture and Forestry, Navsari Agricultural University, Navsari – 396450 (Voucher Specimen No. – SDPC/2011-12/04).

2.2 Preparation of the extract [12]:

The roots of plant were washed with running water, cut in to small pieces and dried under shade. The dried plant material (1 kg) was then crushed and fine powdered. Fine powder was size separated using 44 screen sieve. The powder was extracted using maceration method with methanol. Powder of drug was soaked with methanol for 72hrs. After 72 hrs extract was filtered and evaporated in a water bath evaporator to remove the methanol. The extraction yield was calculated after evaporation of methanol. The extract was collected and stored in air tight glass bottle.

2.3 Phytochemical investigation:

Phytochemical investigation was carried out using CPME for the presence and confirmation of different chemical constituents. The tests were carried out by standard methods described in practical Pharmacognosy by Dr. C. K. Kokate [20] and K. R. Khandelwal [21].

2.4 Experimental Animals:

The experiments were carried out on either sex of Swiss albino mice, 28 – 40 g and either sex of Wistar rats, 220 - 270 g, provided by Shree Dhanvantary Pharmacy College, Kim, Surat. They were maintained under controlled condition of temperature (23 ± 2^{o}C), relative humidity (55 ± 10%), and 12 h / 12 h light / dark cycles. They were randomized into experimental and control groups and housed each in sanitized polypropylene cages containing sterile paddy husk. They had free access to standard pellets as basal diet and water ad libitum. The experiment was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), New Delhi, India and approved by the Animal Ethical Committee of Shree Dhanvantary Pharmacy College, Kim, Surat. (Approval Reg.No:1103/PO/abc/07/cpcsea/CPCSEA and Date- 04-02-2012)

2.5 Drugs and chemicals:

Montelukast, the standard drug for study was provided by Zydus Cadila, Ahmadabad as a gift sample. Dextran Sodium Sulphate was procured from Sigma–Aldrich (St Louis, MO, USA). Acetic acid, Methanol and all other chemicals were of analytical grade provided by Shree Dhanvantary Pharmacy College.

2.6 Acetic acid-induced colitis in rats [22]:

Either sex of Wistar albino rats (220 – 270 g) were selected for the study and randomized into the following groups for treatment:

Group I: Positive control- Acetic acid (1 ml of 4% v/v, intrarectally on 6th day)

Group II: Montelukast (20 mg/kg/day, PO for 5 days) +1 ml of 4% v/v acetic acid (on 6th day)
Group III: CPME (higher200mg/kg/day, PO for 5 days) + 1 ml of 4% v/v acetic acid (on 6th day)
Group IV: CPME (higher 400mg/kg /day, PO for 5 days) + 1 ml of 4% v/v acetic acid (on 6th day)

Animals were pretreated with CPME (200 mg/kg, 400 mg/kg, PO) and Montelukast (20 mg/kg, PO) for 5 days. After the last dose of extract and standard drug animals were fasted over night. On the 6th day animals were anaesthetized using anesthetic ether and 1 ml of 4% v/v acetic acid solution was installed into rectum by intrarectal route for induction of colitis. After 30 seconds of exposure 2 ml of phosphate buffer (pH 6) was injected through the same intrarectal rout. After 48hrs, blood was collected by puncture in retro-orbital plexus. Animals were then sacrificed by cervical dislocation and dissected to remove colon. Waste material was removed from colon and it was flushed with saline gently. Inflammation was assessed based on the macroscopic and microscopic features. Quantification of inflammation was done using biochemical assay, blood examination and histopathological investigation of colon tissue.

2.7 DSS induced colitis in mice [23]:

Either sex of Swiss albino mice (28 - 40 g) were selected to carry out the experiment and randomized into groups for treatment as follows:

Group I : Positive control – DSS (1.5% w/v, PO for 7 days)
Group II: Montelukast (20 mg/kg/day, PO) + DSS (Both given simultaneously for 7days)
Group III: CPME (higher 200mg/kg dose) +DSS (Both given simultaneously for 7days)
Group IV: CPME (higher 400mg/kg dose) +DSS (Both given simultaneously for 7days)

Experimental colitis was induced in swiss albino mice of 8 weeks; by giving drinking water *ad libitum* containing 1.5% w/v DSS. The dose of extract (200 mg/kg and 400 mg/kg, PO) gave parallel with DSS for 7 days in mice. Each day in morning the body weight of mice, stool consistency and gross bleeding were determined for 7 days. On 8th day, the blood was collected from mice by puncture in retro-orbital plexus. Mice were sacrificed by cervical dislocation and dissected to remove colon. Waste material was removed from colon and it was cleaned with saline gently. Inflammation was assessed based on the macroscopic and microscopic features. Quantification of inflammation was done using biochemical assay, blood examination and histopathological investigation of colon tissue of mice.

1.8 Evaluation of the disease:

The disease induced in experimental animals was evaluated based on its macroscopic characteristic, microscopic characteristics and disease activity index. Evaluation pattern for macroscopic characteristics was used after some modifications. The inflammation was quantified using myeloperoxidase assay and blood examination for WBC count, ESR and ALP. Histopathological investigation of intestine tissue was carried out.

2.8.1Evaluation based on macroscopic characters [23]:

(a) Scoring for rat colon:

For each animal, the distal 10 cm portion of the colon was removed and cut longitudinally, and slightly cleaned in physiological saline to remove fecal residues. Pieces of rat ileum and colon (10 cm long each) were scored for macroscopic features using following scoring pattern.

Score of macroscopic changes

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

2.8.2 Evaluation of disease activity index (DAI) [24]:

To evaluate the disease activity index (DAI) the animals were examined every day till the end of experiments. Body weight, stool consistency and gross bleeding of all the rats and mice were recorded daily. DAI was determined by combining scores of (I) body weight loss, (II) stool consistency and (III) gross bleeding, divided by 3.

Each score was determined as follows:

Change in body weight loss

0 none,
1 1–5%,
2 5–10%,
3 10–20%,
4 >20%

Gross bleeding

0 normal
1 hemoccult positive (+)
2 hemoccult positive (++)
3 gross bleeding (+++)
4 gross bleeding (++++)

Stool consistency

0 normal,
1 and 2 loose stool,
3 and 4 diarrhea.

Body weight loss was calculated as that the percent difference between the original body weight (day 0) and the body weight on any particular day (day on which animals were sacrificed).

2.9 Myeloperoxidase activity (MPO) [25]:

The isolated pieces of inflamed tissues (colon, 1 - 2 cm) were taken from different treatment groups. The tissues were then rinsed with saline, blotted dry and weighed. They were individually homogenized in 5ml of phosphate buffer (PH 6). Homogenized tissue was centrifuged at 10,000 rpm for 20 mints. Supernatant collected and mixed with o-phenylenediamine (600 µg/ml in phosphate buffer) and 300mM of H2O2 were used to initiate the reaction. The change in absorbance was measured spectrophotometrically (Shimadzu UV 2450 spectrophotometer), at 492 nm for 5 min. One unit of MPO activity is defined as the change in absorbance per minute by 1.0 at room temperature, in the final reaction.

Calculation of MPO activity:

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

$$\text{Where } X = \frac{\text{10 x changes in absorbance per minute}}{\text{Volume of supernatant taken in the final reaction}}$$

2.10 Evaluation based on microscopic (histological) characters [26]:

At the end of study, the animals will sacrificed by decapitation. The resected large intestine will grossly examine for the mucosal defect, hemorrhage, or ulcerative lesions. Than fixed immediately in 4% neutral formalin. For histopathological analysis, tissue sections was made from the representative region of large intestine by the conventional tissue preparation methods and viewed under the light microscope (100 ×) after hematoxylin and eosin (H&E) staining [116]. Histopathological studies of colons were carried out at "Khandwala Clinical Laboratory Surgical Pathology & Cytology Center", Surat.

2.11Statistical analysis:

All data was expressed as mean ± standard error of the mean (S.E.M.) of 6 rats and 6 mice per experimental group. Statistical analysis was performed using Graph pad prism 5.0 statistical software. Parametric one way analysis of variance (ANOVA) followed by Dunett's post test. The minimal level of significance was identified at $p<0.05$ (95% confidence interval).

3. Results

3.1 Extraction yield:

Cp roots were extracted with Methanol using maceration method of extraction. The extraction yield of this plant part obtained was 1.7% w/w.

3.2 Phytochemical investigation:

Methanolic extract of CP root was investigated for different phytochemical tests and showed positive results for alkaloid, steroid and saponin.

Table 1: Phytochemical test of CPME

Constituents	Test	Result	Inference
Alkaloids	Dragendroff's test	Orange brown precipitate	Positive
	Wagner's test	Reddish brown precipitate	Positive
	Hager's test	Yellow precipitate	Positive
Steroids	Lieberman Burchard test	First red then blue and finally green color appears	Positive
Saponins	Foam test	Foam appears	Positive

3.3 Acetic acid- induced colitis in rats:

Intra-rectal administration of acetic acid caused inflammatory reaction in the colon. The inflammation covered rectum and distal colon portion. The visible changes included severe hyperemia, epithelial necrosis and ulcerated mucosa. These changes were significantly decreased during the treatment with extract and Montelukast**.** Decreasing in the weight loss, gross bleeding and change in stool consistency were also observed in the extract treated groups. Increase in ESR, ALP, WBC counts was significantly ameliorated by the treatment with CP.

Table 2: Effects of CPME on Scoring for macroscopic Evaluation, MPO, DAI, ESR, ALP and WBC count in Acetic acid induced colitis in rats.

Group	Scoring for macroscopic evaluation	MPO (ng/gram tissue)	DAI	ESR (M.M)	ALP (u/l)	WBC (per C.MM)
Group 1	6.16 ± 0.47	1.53 ± 0.12	2.77 ± 0.11	4.83 ± 0.30	244.66 ± 4.65	11700.00 ± 790.36
Group 2	2.83± 0.30***	0.73±0.1***	1.10±0.14***	2.83±0.30***	188.16± 6.17***	7766.67± 477.26***
Group 3	4.33 ± 0.55*	1.180 ± 0.1*	2.16 ± 0.16*	3.50 ± 0.22*	204.83±10.86**	9633.3 3±558.37*
Group 4	3.83 ± 0.47**	0.96 ± 0.05**	1.73 ± 0.26**	3.16 ± 0.30**	192.16 ± 5.38***	8683.33± 357.23**

Each value represents Mean ± S.E.M. (*n* =6). Significant in values according to one-way ANOVA followed by Dunett's t-test. *p<0.05, **p<0.01, ***p<0.001 vs. control group of Acetic acid induced colitis in rats.

3.4 DSS induced colitis in mice:

Seven days treatment with DSS (1.5% w/v) was produces inflammation in the animals of all group. The middle portion of small intestine also shows inflammation when DSS was administered. Ileum and large intestine showed hemorrhagic spots, many lesions, which were transmural. In between there were skip areas of normal tissue. These lesions were decreased in extract treated and standard drug treated group compared to positive control group. DAI, ESR, ALP, WBC counts were also decreased due to treatment of CP extracts.

Table 3: Effects of CPME on Scoring for macroscopic Evaluation, MPO, DAI, ESR, ALP and WBC count in DSS induced colitis in mice.

Group	Scoring for macroscopic evaluation	MPO (ng/gram tissue)	DAI	ESR (M.M)	ALP (u/l)	WBC (per C.MM)
Group 1	4.16 ± 0.30	0.80 ± 0.11	2.27 ± 0.30	4.66 ± 0.42	239.33 ± 7.05	11833.30 ± 1166.10
Group 2	1.50 ±0.42***	0.24 ±0.03***	1.10 ± 0.25***	2.66 ± 0.33**	175.16 ± 6.79***	7583.33 ± 390.23**
Group 3	2.66 ± 0.333*	0.49 ± 0.07*	1.49 ± 0.14*	3.33 ± 0.33*	212.83 ± 4.60**	9266.67 ± 583.48*
Group 4	2.16 ± 0.40**	0.37 ± 0.05**	1.21 ± 0.16**	3.00 ± 0.36*	196.33 ± 6.07***	8550.0 ± 423.28*

Each value represents Mean ± S.E.M. (*n* =6). Significant in values according to one-way ANOVA followed by Dunett's t-test. *p<0.05, **p<0.01, ***p<0.001 vs. control group of DSS induced colitis in mice.

3.5 Myeloperoxidase activity (MPO):

This activity showed significant increase of MPO enzymatic level in positive control group. Increasing in enzyme in tissue indicate abnormal change in body and showed the presence of inflammation. Extract and montelukast treated groups showed significant decrease in MPO activity compared to positive control group in both models.

3.6 Histopathological Observation:

3.6.1 Acetic acid induced colitis in rats:

Histological examination of control group showed massive necrosis of the mucosa and submucosa, crypt infiltration of lymphocytes and eosinophils. Extract and standard drug treated groups showed mild lesions, crypt and villi of colon with decrease inflammation, restricted inflammatory cells and normal functioning colonic mucosa.

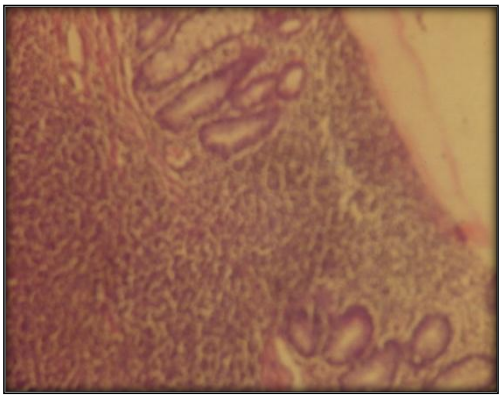

Fig.1 (a). Acetic Acid Induced Colitis

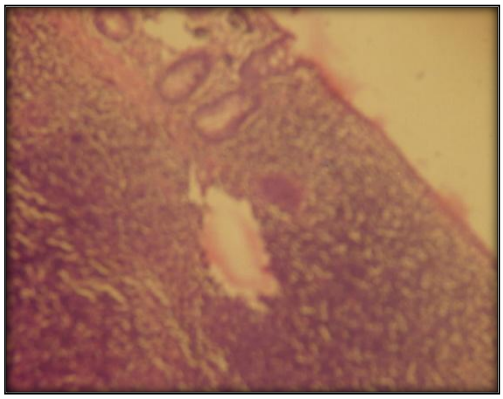

Fig.1 (b). Inflammatory infiltration in CPME lower doses (200 mg/kg)

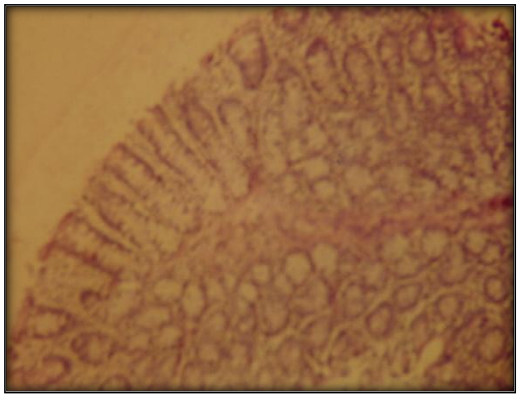

Fig.1 (c). Crypt and Villi in colons with decrease inflammation

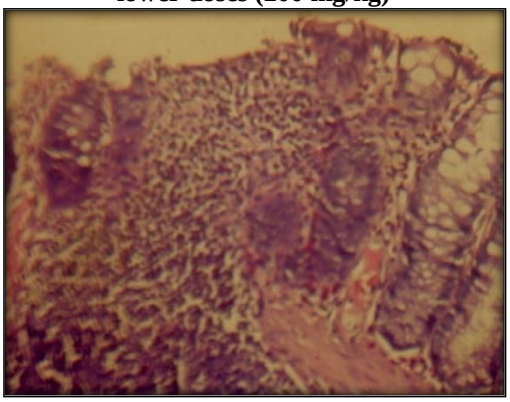

Fig.1 (d). Montelukast treated group

3.6.2 DSS induced colitis in mice:

Histological examination of control group showed moderate inflammation, inflammatory infiltration and enlarges lymphoid follicles mucosa and submucosa. Extract treated groups showed minimal inflammatory cells, mild lymphocytic infiltrate, and near normal colonic mucosa. Montelukast treated group showed suppressed inflammatory reaction**.**

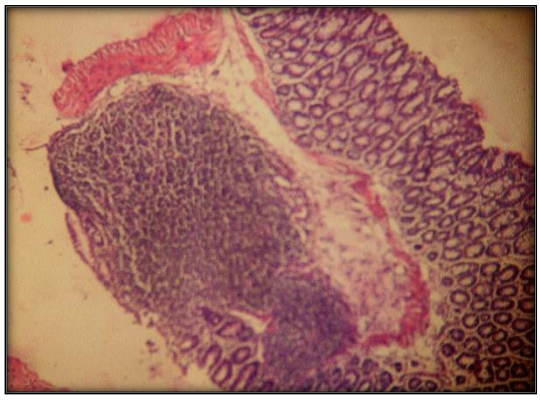

Fig.2 (a). Moderate Inflammation by DSS

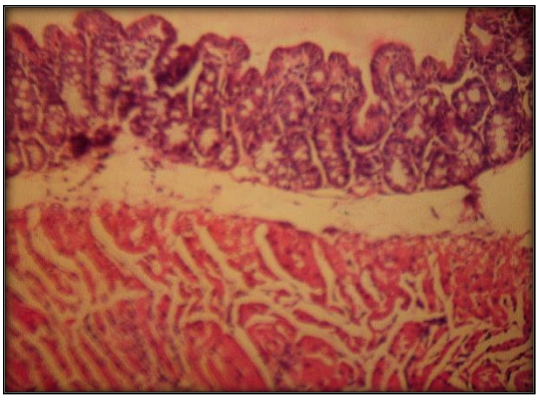

Fig.2 (b). Minimal Inflammatory cell in CPME 200 mg/kg

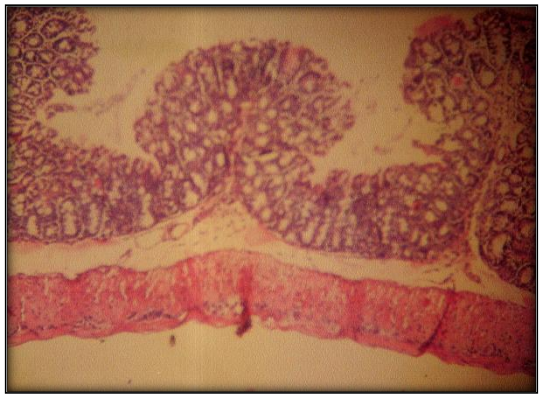

Fig.2 (c). Nearly normal colonic mucosa in CPME 400 mg/kg

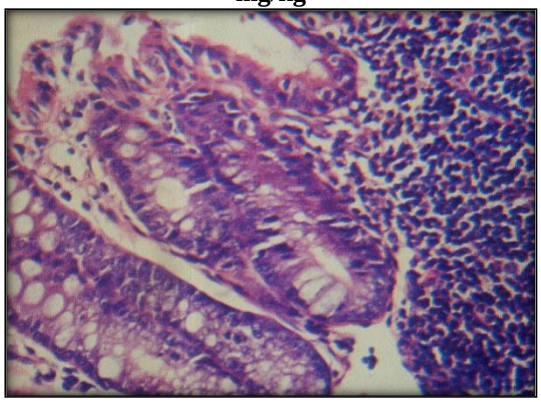

Fig.2 (d). Montelukast treated group

4. Discussion and conclusion:

In acetic acid induced IBD model, intrarectal administration of acetic acid in rats affects, only the distal colon portion. Massive necrosis of mucosal and submucosal layers was observed. This model shares many of the histological features of ulcerative colitis in human beings including epithelial necrosis, mucosal edema, neutrophils infiltration of the mucosa and submucosal ulceration [27]. Dextran sodium sulphate is chemical produces colitis in mice by orally administration. This causes hematochezia, body weight loss, shortening of the intestine, mucosal ulcers, and infiltration of neutrophils. There is more evidence for the involvement of the T-cells in the development of DSS – induced colitis in mice [23].

CP showed the persuasive protective effect against the acute inflammation induced by the carrageenan [18] which is already been reported. In the present investigation, methanolic extract of CP roots showed dose dependent anti-IBD activity in acetic acid induced colitis in rats and DSS induced colitis in mice. These models exhibit symptoms comparable to those of human ulcerative colitis [24], such as body weight loss, diarrhea, bloody feces, and mucosal ulceration [24]. Treatment with CP significantly ameliorates the change in colonic lesion, Disease activity index, myeloperoxidase activity, which showed its protective role in IBD

MPO, a member of the heme peroxidase–cyclooxygenase superfamily is abundantly expressed in neutrophils, and to a lesser extent in monocytes and certain types of macrophages, and thus, is a specific biomarker of inflammation, which was elevated in the present study [28]. Treatment with CP extract reduces the neutrophils infiltration which can be observed by suppression of colon MPO and improvement of histological features.

Other parameters which are directly correlated with the inflammatory condition such as WBC count, ESR, ALP were raised during inflammation and get ameliorated with CP extract.

From the above study, different phytoconstituants in methanolic extract of CP at two different dosage levels

may be responsible for suppression of inflammatory conditions. Since both these models in animals represent UC and CD in humans respectively, we may say that CP may be useful in treatment of above disorders and CP may have an added advantage of minimizing the side effects being an herbal preparation.

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