ORIGINAL ARTICLE

Effectiveness of *Ricinus communis* root extract against dextran sodium sulfate induced ulcerative colitis in rats

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Abstract

Introduction: Ulcerative colitis (UC) is an idiopathic, autoimmune, and inflammatory disorder which affects the lining of the colon and the rectum. It involves inflammatory mediators such as cytokines, nitrogen and oxygen-derived free radicals exerting oxidative stress, deranged colonic milieu, and increase in intestinal permeability. *Ricinus communis* roots are used traditionally in diseases of liver and rectum, in gastropathy such as constipation, inflammation, fever, colic and in colon cancer. Several reports exhibiting analgesic, antibacterial, anti-inflammatory, and antioxidant activity of *R. communis* root extract (RCRE) are available. Hence, this study was undertaken with the objective to evaluate the effectiveness of RCRE in dextran sodium sulfate (DSS) induced UC in rats. **Materials and Methods**: A total of 30 Sprague-Dawley rats were divided into five groups (n = 6). 5- Aminosalicylic acid was used as a standard drug, and RCRE was administered at a dose of 250 mg/kg and 500 mg/kg p.o. for 14 days. UC was induced by replacing drinking water with 4% DSS solution for the past 7 days. **Results**: Severity of colitis induced was assessed by observing macroscopic and microscopic characteristics and changes in the level of oxidative stress parameters. All parameters were altered in model control rats, while pre-treatment with RCRE preserved normal colonic architecture, improved macroscopic and microscopic scores and altered oxidative stress biomarkers in the colon. **Conclusion**: Results showed the good effectiveness of RCRE against DSS-induced UC possibly by its anti-inflammatory and antioxidant activity.

Key words: Anti-inflammatory, dextran sodium sulfate, oxidative stress, *Ricinus communis* root extract, ulcerative colitis

INTRODUCTION

Ulcerative colitis (UC) is an idiopathic, autoimmune, and inflammatory disorder of colon/bowel.[1] It is an inflammatory disease that affects the lining of the colon and the rectum. Pathological findings associated with UC are increased in certain inflammatory mediators including cytokines, nitrogen and oxygen-derived free radicals exerting oxidative stress, deranged colonic milieu, and increase in intestinal permeability.[2,3] Excessive production of nitric oxide (NO) by inducible NO synthetase in chronic colitis may be detrimental to the integrity of mucosa based on generation of reactive nitrogen species (RNS) which causes cellular degeneration in various tissues contributing to the development of intestinal damage, plasma, and mucosal prostaglandin rise simultaneously with degree of colonic injury.[4,5] It is treated using anti-inflammatory drugs, oral steroids, immune suppressants, antibiotics, and anti-tumor necrosis factor-α drugs.[1] All these drugs are typically associated with side effects which limit their usefulness. Plants are considered to be a goldmine to tackle complex inflammatory conditions with minimum side effects. Various plantsare also reported to be useful in IBD due to their immune modulatory antiulcerogenic property and anti-inflammatory property.[6-10] The plant *Ricinus communis* belongs to the family Euphorbiaceae. It is also known as castor in English, erand in Hindi, erendo and ereandi in

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Guajarati. It is a tropical plant distributed widely across the world.[11] Root part of *R. communis* is traditionally claimed as anti-inflammatory for diseases of liver, spleen, pile disorders, diseases of rectum, in gastropathy such as constipation, inflammation, fever, colic and in colon cancer.[12-14] It is also used orally for arthritis and sciatica and as an antipyretic and analgesic.[14] *R. communis* root extract is reported to possess analgesic,[15,16] antibacterial[17] anti-inflammatory, and antioxidant activity.[18,19] 2-5% dextran sodium sulfate (DSS) in drinking water causes hyperemia, ulceration and submucosal edema of the colon, resulting in softening of stool with hemoccult, which symptomatically resembles UC in man.[20-23] Thus, a hypothesis was generated that, a root extract of *R. communis* may be effective in the treatment of UC, which is an inflammatory disease of the bowel/colon with oxidative stress as the main etiology. Literature survey shows that root part of *R. communis* has not been investigated for its effectiveness in UC. Hence, this study was undertaken with the aim of evaluation of *R. communis* root extract (RCRE) in DSS-induced UC in rats.

MATERIALS AND METHODS

Drugs and Chemicals

5- Aminosalicylic acid (5-ASA) procured from Sigma-Aldrich, DSS purchased from MP Biomedicals, Mumbai, India were used for the study. All other chemicals and reagents used were of analytical grade.

Animals

Male Sprague-Dawley rats with an average weight of 200 g were procured from Zydus Research Centre, Ahmedabad. The animals were housed in polypropylene cages at 25±2°C temperature with a relative humidity of 45-55% under 12:12 h light - dark cycles. They were fed with standard laboratory animal feed and water *ad libitum*. The experimental protocol (Protocol no: CPCSEA/IAEC/ARCP/2015-2016/02) was approved by the Institutional Animal Ethics Committee (IAEC) of A. R. College of Pharmacy and G. H. Patel Institute of Pharmacy, Vallabh Vidyanagar, Gujarat, and all experiments were carried out as per CPCSEA guidelines.

Preparation of the Extract

The roots of the plant *R. communis,* Family - Euphorbiaceae were collected from Karjan, District Vadodara, Gujarat and identified and authenticated (Authentication no: AAU/BACA/SST/SSN/215/16) by Dr. Sasidharan N., Head, Department of Seed Science and Technology, B. A. College of Agriculture, Anand, Gujarat. A voucher specimen of the plant is deposited in the botany herbarium of A. R. College of Pharmacy and G. H. Patel Institute of Pharmacy, Vallabh Vidyanagar (Herbarium no: MS/RC-01/30/ARGH-16).

Collected roots of the plant were chopped, shade dried and coarsely powdered. The powder was defatted with petroleum ether (60-80°C) and extracted with methanol using Soxhlet Extractor. The extract was dried.[18] The percentage yield of the extract obtained was 4.2% (w/w). The extract was stored in airtight container at 2-5°C for further experimental use.

Doses equivalent to 250 and 500 mg/kg body weight were calculated and suspended in 0.5% carboxymethyl cellulose (CMC) solution for the experiment.

Preliminary Phytochemical Screening[24]

RCRE was subjected to preliminary phytochemical screening of extract to detect the presence of various active phytoconstituents.

Induction of Colitis in Rats

Colitis was induced in rats by replacing drinking water with 4% DSS solution orally for the past 7 days of 21 days treatment period.[6,20-22,25-27]

Grouping of Animals and Dose Schedule

A total of 30 Sprague-Dawley rats were randomly divided into five groups with six animals in each group. Group I (normal control group) received vehicle (0.5% CMC [1 ml/kg] p.o.) for 3 weeks (21 days). Group II (model control group) received vehicle (0.5% CMC [1 ml/kg] p.o.) for 2 weeks (14 days) followed by 4% DSS in drinking water in the last week (7 days). Group III received 5-ASA (500 mg/kg) p.o. once daily for 14 days. Groups IV and V received RCRE (250 mg/kg and 500 mg/kg p.o., respectively) for 14 days. On day 15, oral administration of 5-ASA and RCRE to Groups III-V was stopped, and intrarectal administration of 5-ASA, RCRE (250 mg/kg), and (500 mg/kg), respectively, was started. Simultaneously colitis was induced in Groups III-V by replacing drinking water with 4% DSS solution for the past 7 days (3rd week). On day 21, rats were sacrificed under anesthesia. The distal 10 cm portion of the colon of all animals of all groups was removed, cut longitudinally, cleaned and used for macroscopic scoring and histopathological scoring. Furthermore, colon length, colon weight, colon weight/length ratio, and oxidative stress parameters were measured.[27]

Assessment of Colitis

Body weight

Body weight of all animals of all groups was recorded on every alternate day and change in body weight at the weekly interval was calculated and documented.[29]

Stool consistency

Stool consistency of all animals of all groups was observed daily and scored on a scale ranging from 0 to 3 as follows: (0) Normal stool, (1) soft stool but still formed, (2) very soft stool, and (3) diarrhea.[29]

Macroscopic score

Isolated colons were examined for signs of inflammation by an independent blind observer. Severity of inflammation was observed macroscopically and scored according to scale ranging from 0 to 10 as follows: (0) No damage, (1) focal hyperemia, (2) ulceration without hyperemia or bowel wall thickening, (3) ulceration with inflammation at 1 site, (4) 2 sites of ulceration and inflammation, (5) major sites of inflammation >1 cm along the organ, and (6-10) major sites of inflammation >2 cm along the organ.[29]

Microscopical analysis

Colons of all animals of all groups were removed, rinsed with phosphate-buffered saline (PBS, pH 7.4) and opened longitudinally. Colon was immersed immediately in neutral buffered 10% formalin, embedded in paraffin and incubated overnight at 4°C. After incubation colon was cut into 5 mm cross sections. The cross sections of colon were stained using Hematoxylin and Eosin stain and were interpreted in a blinded manner by a pathologist using scale ranging from 0 to 4 as follows: (0) No evidence of inflammation, (1) low level of inflammation with scattered infiltrating mononuclear cells, (2) moderate inflammation with multiple foci, (3) high level of inflammation with increased vascular density and marked wall thickening, and (4) maximal severity of inflammation with transmural leukocyte infiltration and loss of goblet cells.[29]

Oxidative stress biomarkers

Remaining portion of the colon was used for assessment of myeloperoxidase (MPO), malondialdehyde (MDA), no level, and superoxide dismutase activity (SOD). MPO activity was calculated by finding out the ratio of change in absorbance and molar extinction coefficient for O-dianisidine.[27] Lipid peroxidation was evaluated by measuring MDA using the thiobarbituric acid method described by Marquez, 2010 and expressed as nmoles of MDA/mg protein. NO level in the colon was measured using the standard curve of 0.001M sodium nitrite in water and reported as nmoles/mg protein.[30] SOD activity was determined and expressed as WU/mg of protein.[31]

Statistical Analysis

All data are expressed as mean±standard error of mean of six rats per experimental group. Statistical analysis was performed using Instat GraphPad 3.0 statistical software. All parametric data were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's test. All non-parametric data were analyzed by non-parametric ANOVA followed by Friedman test. $P < 0.05$ was considered as statistically significant.

RESULTS

Preliminary Phytochemical Screening of RCRE

The preliminary phytochemical screening of methanolic extract of RCRE using standard chemical tests was found to contain carbohydrates, glycosides, saponins, flavonoids, alkaloids, tannins, and phenolic compounds.

Effect of RCRE on Body Weight

Body weight of animals in all groups increased up to 14 days (2 weeks). In the 3rd week, in model control group increase in body weight was significantly less ($P < 0.0001$) due to induction of colitis. Pre-treatment of rats with 5-ASA (500 mg/kg) and RCRE (250 and 500 mg/kg p.o.) for 14 days, significantly increased ($P < 0.001$) body weight as compared to DSS-treated model control rats [Figure 1].

Effect of RCRE on Stool Consistency

Induction of colitis by use of 4% DSS resulted in significant increase ($P < 0.001$) in stool consistency score in model control rats as compared to score of normal rats. 14 days pre-treatment of rats with 5-ASA and RCRE (500 mg/kg) significantly decreased ($P < 0.05$) stool consistency score as compared to score of model control rats. RCRE (250 mg/kg) also reduced stool consistency score, but the difference was not found to be significant [Figure 2].

Effect of RCRE on Morphological Characteristics and Macroscopical Score

Replacing drinking water by 4% DSS for the past 7 days (3rd week) induced colitis of varying severity. Inflammatory reaction in the colon was evidenced by focal hyperemia, bowel wall thickening, and inflammation along the length of the colon. Colon of 5-ASA and RCRE treated groups showed signs of suppression of inflammatory reaction with mild lesions [Figure 3].

The mean macroscopical score in model control rats was significantly higher ($P < 0.01$) as compared to normal control rats. Pre-treatment with 5-ASA and RCRE (250 and 500 mg/kg) (500 mg/kg) in rats for 14 days, significantly ($P < 0.01$) and dose-dependently decreased macroscopic score as compared to DSS-treated model control rats [Table 1].

Effect of RCRE on Microscopical Score

As compared to normal control group, the mean microscopically score in model control rats was significantly increased ($P < 0.001$). The 14 days pre-treatment with 5-ASA prevented mucosal damage and significantly decreased ($P < 0.05$) mean microscopically score when compared to

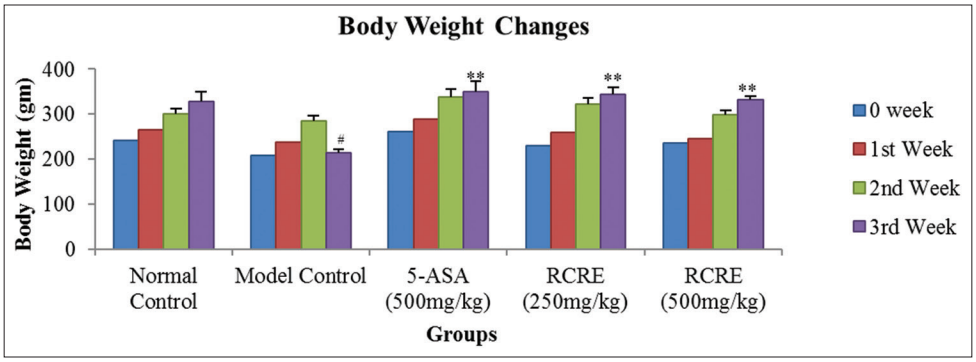

Figure 1: Effect of *Ricinus communis* root extract (250 and 500 mg/kg) and 5- Aminosalicylic acid (500 mg/kg) on body weight changes in rats at the end of 3 weeks as compared with model control rats. ***P*<0.000, #*P*<0.001

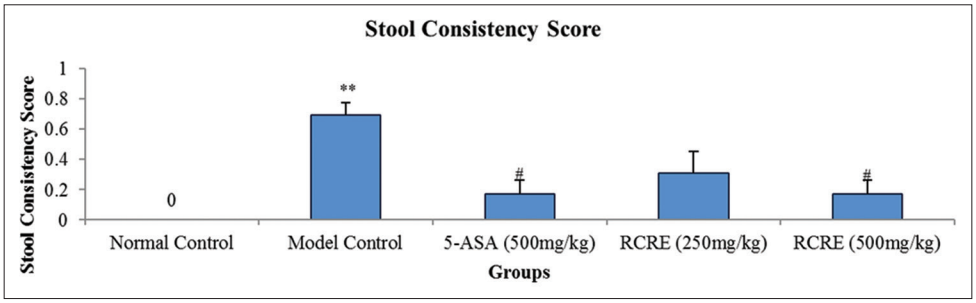

Figure 2: Effect of *Ricinus communis* root extract (500 mg/kg) and 5- Aminosalicylic acid (500 mg/kg) on stool consistency score as compared with normal control rats (**) and model control rats (##). ***P*<0.001, #*P*<0.05

model control group. Similar results were obtained with RCRE (250 and 500 mg/kg) treated groups, where mucosal damage and inflammation was prevented by RCRE resulting in significant and dose-dependent reduction ($P < 0.05$) in the mean microscopical score when compared with DSS-treated model control rats [Table 1 and Figure 4].

Effect of RCRE on Colon Length, Colon Weight

Mean colon length and colon weight in DSS-treated model control rats were significantly increased ($P < 0.01$) as compared to normal rats suggesting induction of colitis by DSS. Comparing DSS-treated model control rats with RCRE (500 mg/kg) treated rats, the increase in mean colon length was significantly less ($P < 0.05$). Furthermore, significantly less increase in colon weight was observed in RCRE (250 and 500 mg/kg) treated rats as compared to model control rats ($P < 0.05$ and $P < 0.01$, respectively). Similar results were observed in rats pre-treated with 5-ASA (500 mg/kg) ($P < 0.01$) [Table 1].

Effect of RCRE on Colon Weight to Length Ratio

Colon weight to length ratio in model control rats increased due to induction of colitis by DSS. Pre-treatment of rats with 5-ASA (500 mg/kg) and RCRE (250 and 500 mg/kg) for 14 days decreased colonic weight to length ratio as compared to DSS-treated model control rats. However, the difference was not found to be statistically significant [Table 1].

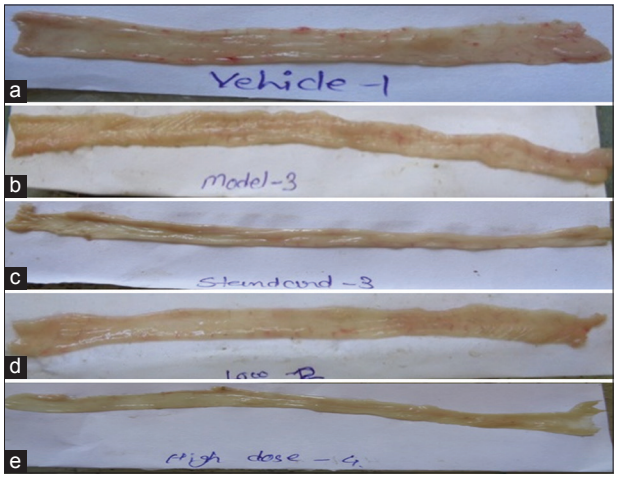

Figure 3: Morphological features of colons from rats of various groups. Colon morphology images: (a) Normal rat colon, (b) dextran sodium sulfate treated model control rat colon, (c) 5- Aminosalicylic acid (500 mg/kg) treated rat colon, (d) *Ricinus communis* root extract (RCRE) (250 mg/kg) treated rat colon, and (e) RCRE (500 mg/kg) treated rat colon

Effect of RCRE on Levels of Oxidative Stress Parameters

MPO content and NO level in DSS-treated model control rats were significantly ($P < 0.0001$) increased as compared normal rats. The 14 days pre-treatment with 5-ASA (500 mg/kg) and RCRE (250 mg/kg and 500 mg/kg p.o.) significantly

Table 1: Effect of RCRE (250 and 500 mg/kg) and 5-ASA (500 mg/kg) on colonic macroscopic score, microscopical score, colon length, colon weight, and weight to length ratio

Groups	Macroscopic score	Microscopical score	Colon length	Colon weight	Colon Wt/L
Normal control	0	0	8.78±0.24	0.94±0.07	0.107±0.01
Model control	7.3±0.42**	2.5±1.02**	11.8±0.36##	1.36±0.10**	0.115±0.104
5-ASA (500 mg/kg)	1.83±0.30**	0.5±0.20#	9.03±0.30##	1.01±0.02##	0.111±0.004
RCRE (250 mg/kg)	2.5±0.34**	1.5±0.61	10.8±0.40	1.07±0.04#	0.106±0.004
RCRE (500 mg/kg)	2±0.36**	1±0.40#	9.88±0.29#	1.05±0.04##	0.099±0.006

**P<0.001, ##P<0.01, #P<0.05, RCRE: *Ricinus communis* root extract, 5-ASA: 5- Aminosalicylic acid, *R. communis: Ricinus communis*

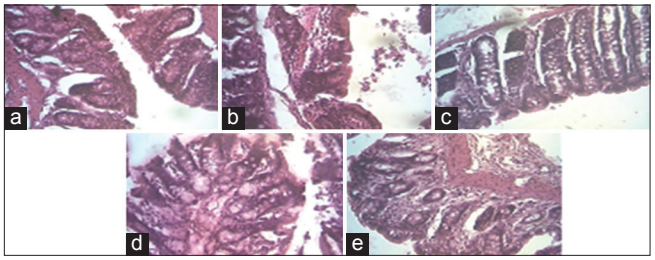

Figure 4: Photomicrographs of sections of colons from rats of various groups stained with H and E. Colon microscopic image: (a) Normal rat with normal mucosal morphology, (b) dextran sodium sulfate induced colitis rats with extensive mucosal damage and inflammation, (c) 5- Aminosalicylic acid (500 mg/kg) treated rats with mild mucosal damage and inflammation, (d) *Ricinus communis* root extract (RCRE) (250 mg/kg) treated rats with mucosal damage and inflammation, and (e) RCRE (500 mg/kg) treated rats with mild mucosal damage and inflammation

($P < 0.0001$) prevented the increase in MPO and NO level as compared to model control rats.

Furthermore, pre-treatment with 5-ASA and RCRE (250 and 500 mg/kg) prevent an increase in MDA level as compared to model control rats. However, the difference was not found to be statistically significant.

Treatment with DSS significantly decreased ($P < 0.01$) SOD activity in model control rats, which was significantly ($P < 0.01$) increased by pre-treatment with 5-ASA (500 mg/kg) and RCRE (500 mg/kg). However, the results with RCRE (250 mg/kg) were not found to be statistically significant [Table 2].

DISCUSSION

UC is a chronic, idiopathic, and relapsing disease that causes inflammation and ulceration of colonic mucosa. Its etiology remains undetermined but results from many studies suggest that it is related to an abnormal immune response in the gastrointestinal tract possibly associated with genetic and environmental factors.[32] Pathological findings associated with UC are increased in certain inflammatory mediators including cytokines, nitrogen and oxygen-derived free radicals, signs of oxidative stress, deranged colonic milieu, and increase in intestinal permeability.[2,3]

In this investigation, the protective effect of RCRE was evaluated against DSS-induced colitis in rats. DSS induced colitis is a reproducible model that morphologically and symptomatically resembles UC in humans.[23,33] Some researchers have reported that the use of 2-5% DSS (w/v) (50,000 Da) in drinking water for a period of 5-9 successive days induces colitis in Sprague-Dawley or Wistar rats.[20-22,26] It induces colitis by causing erosion with complete loss of surface epithelium. It causes deformity in the epithelial integrity, thereby increasing colonic mucosal permeability.[20,22,23] DSS-induced colitis causes morphological, histological and macroscopical changes characterized by hyperemia, ulcerations, moderate to severe submucosal edema, lesions, infiltration of granulocytes, and soft stool along with hemoccult.[21,34] DSS also significantly increases the production of all pro-inflammatory cytokines in both mid and distal colon.[35]

RCRE treatment results in improvement of DSS-induced colitis in rats by ameliorating stool consistency and improving body weight when compared to DSS-treated model control rats.

In this study UC-induced by DSS was associated with inflammation along with macroscopic, microscopic, and biochemical changes. By virtue of its healing property, administration of RCRE improved the histological score by ameliorating the disruption of colonic architecture. It also improved macroscopical score as evidenced by attenuation of mucosal damage and wall thickening as compared to DSS control rats.

Increase in weight and length of colon along with elevated weight/length ratio produced by DSS reflect the degree of local inflammation, edema, and wall thickening. The weight of the colon tissue is elevated due to an inflammatory response which is indicative of severity and extent of the disease.[36] All these parameters are significantly decreased by RCRE, suggesting its anti-inflammatory activity.

Several experimental studies have demonstrated that increased infiltration of leukocytes into the colonic mucosa contributes

Table 2: Effect of RCRE (250 and 500 mg/kg) and 5-ASA (500 mg/kg) on colonic oxidative parameters such as, myeloperoxidase content, malondialdehyde level, nitric oxide level, and superoxide dismutase activity

Groups	MPO (U/mg protein)	MDA (nM/g protein)	NO (nM/mg protein)	SOD (U/mg protein)
Normal control	10.74±4.40	0.96±0.39	1.27±0.56	100±40.98
Model control	47.20±19.34**	4.45±1.82	2.68±1.20**	39.03±5.99#
5-ASA (500 mg/kg)	12.08±4.95**	2.02±0.83	1.79±0.80**	92.69±37.98#
RCRE (250 mg/kg)	21.21±8.69**	3.81±1.56	2.47±1.10**	43.91±17.99
RCRE (500 mg/kg)	13.94±5.71**	3.49±1.43	2.07±0.92**	85.37±34.98#

**P<0.0001, #P<0.01, RCRE: *Ricinus communis* root extract, 5-ASA: 5- Aminosalicylic acid, MPO: Myeloperoxidase, MDA: Malondialdehyde, *R. communis: Ricinus communis,* SOD: Superoxide dismutase, NO: Nitric oxide

significantly to the tissue necrosis and mucosal dysfunction, as they represent a major source of reactive oxygen species (ROS) including superoxide, hydrogen peroxide, and hypochlorous acid, which induce oxidative stress.[37,38] ROS attack the cellular macromolecules, thus disrupting epithelial integrity and hindering mucosal recovery, especially in the case of impaired endogenous defense systems.[39] Excessive generation of ROS leads to various pathological processes such as inflammation.

MPO is a good marker of neutrophil infiltration, inflammation and tissue injury. Elevated level of MPO in colonic tissue resulted in the production of superoxide anion, which contributed to the tissue necrosis and mucosal dysfunction in DSS control rats.[40,41] RCRE significantly attenuated this elevated level of MPO by virtue of its antioxidant property.

Increased level of MDA as a marker of lipid peroxidation in association with higher MPO activity confirms the role of free radicals in DSS-induced colitis.[42] Reduction in MDA level in RCRE treated rats shows its protective effect against DSS-induced colitis.

Colonic nitrite level serves as a sensitive marker of disease activity and toxicity in colitis.[43] NO is an unconventional intracellular messenger, playing a key role in various pathological and physiological processes. It interacts with ROS, resulting in cellular damage.[44] Pre-treatment with RCRE restores the elevated level of colonic NO and protects colonic mucosa from damage.

SOD plays an important role in protecting cells from oxidative damage by converting superoxide (O2-) into H_2O_2. In the case of SOD deficiency or increased superoxide production, O^{2-} reacts with NO to produce RNS, such as peroxynitrite ($ONOO^-$), which can cause oxidative/nitrosative stress.[45] Several studies have reported that administration of SOD, suppresses DSS and TNBS induced colitis by decreasing ROS level in the colon.[46,47] Our results are in correlation with these studies, indicating RCRE suppresses the DSS-induced colitis through enhancement of antioxidant functions in the colon.

Wide ranges of phytoconstituents are responsible for the anti-inflammatory and antioxidant activity including phenolic compounds, alkaloids, tannins, and saponins.[49] Several mechanisms have been proposed to explain the anti-inflammatory action of phytoconstituents: (i) Antioxidative and free radical scavenging activities, (ii) modulation of cellular activities of inflammation related to cells, (iii) modulation of pro-inflammatory enzyme activities, and (iv) modulation of pro-inflammatory gene expression.[49-56] Phytochemical screening of the RCRE revealed the presence of carbohydrate, glycosides, saponins, flavonoids, alkaloids, tannins, and phenolic compounds. These phytoconstituents have been found to be effective in various models of IBD and may explain the antioxidant and anti-inflammatory activity of RCRE.

CONCLUSION

From the results of our experiments, we conclude that RCRE in the dose of 500 mg/kg exerts a protective effect against DSS-induced UC in Sprague-Dawley rats by its anti-inflammatory and free radical scavenging or antioxidant activity more or less comparable to standard drug 5-ASA (500 mg/kg). This lends pharmacological support to folkloric, ethnomedical uses of this plant in the management of UC, which is an inflammatory disease of the colon with oxidative stress as the main etiology.

REFERENCES

1. Roger W, Cate W. Clinical Pharmacy and Therapeutics. 5th ed. New York: Churchill and Livingstone; 2012. p. 185-204.
2. Shanahan F. Probiotics and inflammatory bowel disease: Is there a scientific rationale? Inflamm Bowel Dis 2000;6:107-15.
3. Rutgeerts P, Geboes K. Understanding inflammatory bowel disease - The clinician's perspective. Eur J Surg Suppl 2001;586:66-72.
4. Kim JM, Jung HY. Mitogen-activated protein kinase and activator protein-1 dependent signals are essential for *Bacteroides fragilis* enterotoxin induced enteritis. Eur J Immunol 2005;35:2648-57.
5. Wiercinska-Drapalo A, Flisiak R, Prokopowicz D.

Mucosal and plasma prostaglandin E2 in ulcerative colitis. Hepatogastroenterology 1999;46:2338-42.
6. Singh K, Jaggi AS, Singh N. Exploring the ameliorative potential of *Punica granatum* in dextran sulfate sodium induced ulcerative colitis in mice. Phytother Res 2009;23:1565-74.
7. Deshmukh CD, Veeresh B. Protective effect of *Emblica officinalis* fruit extract on acetic acid induced colitis in rats. J Herb Med Toxicol 2010;4:83-7.
8. Kazi HA, Qian Z. Crocetin reduces TNBS-induced experimental colitis in mice by downregulation of NFkB. Saudi J Gastroenterol 2009;15:181-7.
9. Minaiyan M, Ghannadi A. Anti-ulcerogenic effect of (rhizome of *Zingiber officinale* roscoe) hydroalcoholic extract on acetic acid-induced acute colitis in rats. Res Pharm Sci 2008;3:15-22.
10. Alok PC. Effect of Coconut Oil on Ulcerative Colitis in the Mouse Model [Dissertation], Western Kentucky University; 2013.
11. Ladda PL, Kamthane RB. *Ricinus communis* (castor): An overview. Int J Res Pharmacol Pharmacother 2014;3:136-4.
12. Kirtikar KR, Basu BD. Indian Medicinal Plants. 2nd ed., Vol. III. Dehradun: International Book Distributor; 2005. p. 2274.
13. Duke JA. Handbook of Medicinal Herb. 2nd ed. New York, Boca Raton: CRC Press; 2002. p. 161.
14. Ross IA. Medicinal Plant of World. Vol. II. Totowa: Humana Press; 2001. p. 377-8, 380-2.
15. Anilkumar M. Ethnnomedicinal plants as anti-inflammatory and analgesic agents. Ethnomed Source Complement Ther 2010;661:267-93.
16. Taur DJ, Waghmare MG, Bandal RS, Patil RY. Antinociceptive activity of *Ricinus communis* L. Leaves. Asian Pac J Trop Biomed 2011;1:139-41.
17. Islam T, Bakshi H. Assessment of antibacterial potential of leaves of *Ricinus communis* against pathogenic and dermatophytic bacteria. Int J Pharm Res Dev 2010;1:1-7.
18. Ilavarasan R, Mallika M, Venkataraman S. Anti-inflammatory and free radical scavenging activity of *Ricinus communis* root extract. J Ethnopharmacol 2006;103:478-80.
19. Rana M, Dhamija H. *Ricinus communis* L. - A review. Int J PharmTech Res 2012;4:1706-11.
20. Moncada DM, Kammanadiminti SJ, Chadee K. Mucin and toll-like receptors in host defense against intestinal parasites. Trends Parasitol 2003;19:305-11.
21. Dharmani P, Leung P, Chadee K. Tumor necrosis factor-a and Muc2 mucin play major roles in disease onset and progression in dextran sodium sulphate-induced colitis. PLoS One 2011;6:e25058.
22. Cheah KY, Bastian SE, Acott TM, Abimosleh SM, Lymn KA, Howarth GS. Grape seed extract reduces the severity of selected disease markers in the proximal colon of dextran sulphate sodiuminduced colitis in rats. Dig Dis Sci 2013;58:970-7.
23. Gaudio E, Taddei G, Vetuschi A, Sferra R, Frieri G, Ricciardi G, *et al.* Dextran sulfate sodium (DSS) colitis in rats: Clinical, structural, and ultrastructural aspects. Dig Dis Sci 1999;44:1458-75.
24. Harborne JB. Phytochemical Methods. 1st ed. London: Chapman and Hall Ltd.; 1973. p. 4, 34, 109, 140, 168, 182, 243.
25. Ivan C. Inventor. Sigmoid Pharma Limited, Assignee. Compositions for Use in the Treatment of Ulcerative Colitis, WIPO Patent WO2015104414, 16 July; 2015.
26. Dai C, Zheng CQ. VSL#3 probiotics exerts the anti-inflammatory activity via PI3k/Akt and NF-κB pathway in rat model of DSS-induced colitis. Mol Cell Biochem 2013;374:1-11.
27. Márquez L, Pérez-Nievas BG, Gárate I, García-Bueno B, Madrigal JL, Menchén L, *et al.* Anti-inflammatory effects of *Mangifera indica* L. Extract in a model of colitis. World J Gastroenterol 2010;16:4922-31.
28. Wirtz S, Clemens N. Chemically induced mouse models of intestinal inflammation. Lab Immunol 2007;2:541-6.
29. Bobin-Dubigeon C, Collin X. Effects of tumour necrosis factor-alpha synthesis inhibitors on rat trinitrobenzene sulphonic acid-induced chronic colitis. Eur J Pharmacol 2001;431:103-10.
30. Camacho-Barquero L, Villegas I, Sánchez-Calvo JM, Talero E, Sánchez-Fidalgo S, Motilva V, *et al.* Curcumin, a *Curcuma longa* constituent, acts on MAPK p38 pathway modulating COX-2 and iNOS expression in chronic experimental colitis. Int Immunopharmacol 2007;7:333-42.
31. Brody LL, Powell SR. Increased oxidative stress and decreased antioxidant defenses in mucosa of inflammatory bowel disease. Dig Dis Sci 1996;41:2078-86.
32. Elson CO, Sartor RB, Tennyson GS, Riddell RH. Experimental models of inflammatory bowel disease. Gastroenterology 1995;109:1344-67.
33. Jurjus AR, Khoury NN, Reimund JM. Animal models of inflammatory bowel disease. J Pharmacol Toxicol Methods 2004;50:81-92.
34. Ni J, Chen SF, Hollander D. Effects of dextran sulphate sodium on intestinal epithelial cells and intestinal lymphocytes. Gut 1996;39:234-41.
35. Andújar I, Ríos JL, Giner RM, Miguel Cerdá J, Recio Mdel C. Beneficial effect of shikonin on experimental colitis induced by dextran sulfate sodium in BALB/c mice. Evid Based Complement Alternat Med 2012;2012:271606.
36. Rachmilewitz D, Simon PL, Schwartz LW, Griswold DE, Fondacaro JD, Wasserman MA. Inflammatory mediators of experimental colitis in rats. Gastroenterology 1989;97:326-37.
37. Piechota-Polanczyk A, Fichna J. Review article: The role of oxidative stress in pathogenesis and treatment of inflammatory bowel diseases. Naunyn Schmiedebergs Arch Pharmacol 2014;387:605-20.
38. Yoshida N, Yoshikawa T. A novel water-soluble vitamin E derivative protects against experimental colitis in rats. Antioxid Redox Signal 1999;1:555-62.

39. Buffinton GD, Doe WF. Depleted mucosal antioxidant defences in inflammatory bowel disease. Free Radic Biol Med 1995;19:911-8.
40. Baker SS, Campbell CL. Rat enterocyte injury by oxygen-dependent processes. Gastroenterology 1991;101:716-20.
41. Kettle AJ, Gedye CA, Winterbourn CC. Mechanism of inactivation of myeloperoxidase by 4-aminobenzoic acid hydrazide. Biochem J 1997;321:503-8.
42. Herías MV, Koninkx JF, Vos JG, Huis in't Veld JH, van Dijk JE. Probiotic effects of *Lactobacillus casei* on DSS-induced ulcerative colitis in mice. Int J Food Microbiol 2005;103:143-55.
43. Rachmilewitz D, Eliakim R. Direct determination of colonic nitric oxide level - A sensitive marker of disease activity in ulcerative colitis. Am J Gastroenterol 1998;93:409-12.
44. Gow AJ, Farkouh CR, Munson DA, Posencheg MA, Ischiropoulos H. Biological significance of nitric oxide-mediated protein modifications. Am J Physiol Lung Cell Mol Physiol 2004;287:L262-8.
45. Seril DN, Liao J, Yang GY, Yang CS. Oxidative stress and ulcerative colitis-associated carcinogenesis: Studies in humans and animal models. Carcinogenesis 2003;24:353-62.
46. Ishihara T, Tanaka K, Tasaka Y, Namba T, Suzuki J, Ishihara T, *et al.* Therapeutic effect of lecithinized superoxide dismutase against colitis. J Pharmacol Exp Ther 2009;328:152-64.
47. Segui J, Gironella M, Sans M, Granell S, Gil F, Gimeno M, *et al*. Superoxide dismutase ameliorates TNBS-induced colitis by reducing oxidative stress, adhesion molecule expression, and leukocyte recruitment into the inflamed intestine. J Leukoc Biol 2004;76:537-44.
48. Arya V, Arya ML. A review on anti-inflammatory plants barks. Int J PharmTech Res 2011;3:899-908.
49. Oz HS, Chen TS, McClain CJ, de Villiers WJ. Antioxidants as novel therapy in a murine model of colitis. J Nutr Biochem 2005;16:297-304.
50. Mazzon E, Muià C, Paola RD, Genovese T, Menegazzi M, De Sarro A, *et al.* Green tea polyphenol extract attenuates colon injury induced by experimental colitis. Free Radic Res 2005;39:1017-25.
51. Clinton CN. Plant tannins: A novel approach to the treatment of ulcerative colitis. Nat Med 2009;1:1-4.
52. Mueller D, Triebel S, Rudakovski O, Richling E. Influence of triterpenoids present in apple peel on inflammatory gene expression associated with inflammatory bowel disease (IBD). Food Chem 2013;139:339-46.
53. Sener TE, Kardas RC. The effect of betulinic acid on TNBS-induced experimental colitis. Marmara Pharm J 2013;17:52-9.
54. Mochizuki M, Hasegawa N. Antiinflammatory effect of extract of *Terminalia serica* roots in an experimental model of colitis. J Health Sci 2007;53:329-31.
55. Jeong JJ, Jang SE, Joh EH, Han MJ, Kim DH. Kalopanaxsaponin B ameliorates TNBS-induced colitis in mice. Biomol Ther (Seoul) 2012;20:457-62.
56. Joh EH, Kim DH. Kalopanaxsaponin A ameliorates experimental colitis in mice by inhibiting IRAK-1 activation in the NF-kB and MAPK pathways. Br J Pharmacol 2011;162:1731-42.

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