1. ABSTRACT

OBJECTIVE:

The present study was designed to evaluate the effects of *Holarrhena antidysenterica* and *Cyperus rotundus* on DNBS induced IBD in experimental rats as well as to clinically evaluate monoherbal formulation containing extracts of *Holarrhena antidysenterica* in ulcerative colitis patients.

METHOD:

The experimental protocol (Protocol No. 9012 dated 26th Dec 2009) was approved by Institutional Animal Ethical Committee of Anand Pharmacy College. Phytochemical tests and preliminary pharmacological activities of hydromethanolic whole extracts of *Holarrhena antidysenterica* (200, 400 and 600 mg/kg) and *Cyperus rotundus* (300, 500 and 800 mg/kg) was studied in DNBS induced inflammatory bowel disease in rats. Followed by phytochemical tests and efficacy studies of various extracts of *Holarrhena antidysenterica* (600 mg/kg) and *Cyperus rotundus* (800 mg/kg) obtained successively using solvents having different polarity. The safety of the herbal extracts was established through subacute toxicity studies as per OECD guidelines.

Colitis was induced with DNBS (180mg/kg in 50% ethanol) intracolonically in animals on the 11th day of the study. Animals were fasted for 24 hrs prior to study, given access to water *ad libitum*. Rats were treated for 18 days with extracts of *Holarrhena antidysenterica* and *Cyperus rotundus* obtained successively using various solvents for preliminary pharmacological studies.

Sprague Dawley rats were randomly allocated to 9 groups for the main studies. Group I served as the normal control. Group II served as vehicle control which received 50% ethanol intracolonically on 11th day of the study. Group III served as Model control. Animals of Group IV to IX were given the Standard drug 5-Amino Salicylic Acid (5-ASA) (100mg/kg), hydromethanolic extract of *Holarrhena antidysenterica* (MEHA) (450 mg/kg), MEHA (600 mg/kg), chloroform extract of *Cyperus rotundus* (CHCR) (600 mg/kg), CHCR (800 mg/kg) and CMC suspension respectively for 18 days once a day orally. Colitis was induced in animals of Group III-IX. During the study body weight, total food intake & water intake of each group was measured daily.
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Stool consistency was measured for each group daily after induction & scored from 0 to 2, the average score was calculated. On 18th day, blood was collected for the estimation of cortisol. The colon was removed from euthanized rats, length and weight of it was measured. Colon tissue from groups I, II, III, IV, VI and VII were put immediately in tissue collection vials for gene expression studies. Colon from all the groups was stored in the formalin (10%) for the histopathology study and microscopic scoring. Remaining colon tissues were opened along the antimesenteric border and scored for macroscopic parameters like Colon mucosal Disease index (CMDI). Colon homogenate was prepared and was used for estimation of various biochemical parameters Nitric oxide (NO), Malondialdehyde (MDA), Myeloperoxidase (MPO) & Superoxide dismutase (SOD).

Monoherbal formulation was prepared using extracts of *Holarrhena antidysenterica* and its efficacy was compared with Modern (Allopathic) treatment in patients with chronic ulcerative colitis. The study design was randomized and parallel group. The protocol was approved by Institutional Human Research Ethics Committee of Anand Pharmacy College on 23rd Jan 2013. Three groups each of 10 patients were treated with standard Allopathic drug Mesalamine (Mesalazine) (Group I), test drug monoherbal tablet (Group II) and combination of both (Group III) respectively. Baseline characteristics of the patients enrolled, factors affecting their quality of life, chronicity of symptoms and disease before treatment, effect of treatments on signs and symptoms, body weight and various laboratory investigations were recorded. Patients’ compliance, reports of side effects and complications developed, if any was also recorded during the study and follow up visits.

RESULT:

Phytochemical tests confirmed that hydromethanolic whole extracts of *Holarrhena antidysenterica* (WMEHA) contained saponin, steroid, alkaloids, tannins and flavonoids while hydromethanolic whole extracts of *Cyperus rotundus* (WMECR) contained steroids, tannins, alkaloids and carbohydrates. In preliminary pharmacological activity studies, WMEHA (200, 400 and 600 mg/kg) and WMECR (300, 500 and 800 mg/kg) were found to be efficacious in DNBS induced inflammatory bowel disease in rats.
Hydromethanolic extract of *Holarrhena antidysenterica* (MEHA) showed the presence of flavonoids, alkaloids and tannins and chloroform extract of *Cyperus rotundus* (CHCR) showed the presence of steroids in phytochemical tests. MEHA (600 mg/kg) and CHCR (800 mg/kg) were found out to be the most efficacious extracts in efficacy studies done with extracts obtained successively using various solvents.

*Holarrhena antidysenterica* hydromethanolic plant extract was found to be safe up to 1350mg/kg dose while *Cyperus rotundus* chloroform extract did not exhibit any adverse effects upto 1800 mg/kg dose in male and female rats. Maximum efficacy was observed with MEHA and CHCR treated rats.

DNBS Model control animals showed significant reduction in percent change in body weight, water intake, food intake, SOD activity, colon length and significant increase colon weight, MDA, MPO activity, NO, CMDI, DAI & serum Cortisol level. Further expression level of proinflammatory cytokines IL-4, IL-6, IL-12 and IFN-gamma were up regulated in the IBD model control rats and pretreatment with 5-ASA (100mg/kg), MEHA (450 mg/kg) , MEHA (600 mg/kg) , CHCR (600 mg/kg) , CHCR (800 mg/kg), significantly prevented these changes induced by DNBS. The treatments reduced the oxidative stress due to its antioxidant effect and downregulated the transcription of IL-4 mRNA and re-affirmed the reported findings. Increased production of IL-4 in IBD may contribute to disease pathogenesis and its suppression reverses the condition. Increased IL-6 levels in the colonic tissue support their role in the immunopathogenesis of this disorder. Cytokine expression after the treatment with standard drug and investigational herbal extracts decreased when compared to the rats treated with DNBS alone. DNBS led to enhanced expression of mRNA of IL-12 (1.00) and aggravated colon injury. The expression significantly decreased after 5-ASA, MEHA and CHCR administration (0.23, 0.509 and 0.424) in DNBS-induced colitis model, there is direct stimulation of immune cells leading to direct T\textsubscript{H1}-cell response which can be controlled only by direct abrogation of IFN-γ production with exogenous administration of MEHA and CHCR extracts.

In our studies, male preponderance towards UC was observed. Most of the patients were in the age group of 31-40 and 51-60 years. 30% patients were smokers and 70% were leading stressful life. More than 55% patients were chronically found to be
suffering from UC. Patients suffering from several years with chronic diarrhoea responded positively to the trial treatments. All the patients showed positive result for occult blood test done in stool samples which reversed significantly in all the treatment groups. There was 1gm% rise in hemoglobin levels in all the treatment groups when compared to hemoglobin levels before treatment. The treatment with test drug reduced the high scores for signs and symptoms for ulcerative colitis in all smokers as well as nonsmokers. Patients treated with monoherbal tablets alone showed maximal reduction in abdominal pain, diarrhoea, and bowel frequency per day and stool consistency scores and the results were better than Mesalamine treated patients. Treatment with Monoherbal tablet alone and in combination with Mesalamine significantly reduced the stool infection while Mesalamine alone could not resolve the infection. Thus, the monoherbal test formulation alone and in combination with Mesalamine showed significant improvement in combating the clinical symptomatology of IBD. All the treatments significantly reduced the cumulative scores for abdominal pain, diarrhea, stool consistency and bowel frequency/day in all the patients irrespective of their smoking habit, type of stress, age, food habits or chronicity of symptoms.

Patients treated with monoherbal therapy and combination therapy did not report any side effects, relapse or complications while 50% patients treated with Mesalamine exhibited the relapse of the symptoms like diarrhea and flatulence after drug withdrawal. Thus, the efficacy observed in ulcerative colitis patients treated with monoherbal formulation containing extracts of Holarrhena antidysenterica and the group treated with both herbal and allopathic drugs was found to be better than Modern drug Mesalamine (Allopathic) treatment.

CONCLUSION: MEHA and CHCR were found to have anti-inflammatory and antioxidant activities which was beneficial to reduce the severity of the IBD induced by DNBS intracolonically administration in rats. The molecular mechanism behind their efficacy was further substantiated with gene expression studies in which the treated rats showed decreased fold of expression of proinflammatory cytokines IL-4, IL-6, IL-12 and IFN-γ. In clinical studies, monoherbal test formulation when given alone and in combination with standard drug significantly resolved the symptoms of ulcerative colitis when compared to standard allopathic drug. Thus, Holarrhena
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*Holarrhena antidysenterica* and *Cyperus rotundus* is effective, safe and economical and thus can be used as an alternative for the treatment of IBD patients.

**KEYWORDS:** *Holarrhena antidysenterica, Cyperus rotundus,* inflammatory bowel disease, ulcerative colitis.