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RESEARCH ARTICLE

Effect of *Tribulus terrestris* against Inflammatory Bowel Disease

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ABSTRACT

Colitis was induced in animals of groups G2 to G6 by adding Dextran sulfate sodium to drinking water from day 1 to day 7 *ad libitum*. Animals of group G1 received distilled water alone. *T. terrestris,* extract dissolved in distilled water was administered to three groups at the dose levels of 40, 80 and 160 mg/kg body weight respectively. A daily clinical assessment was performed in all the animals, including body weight, evaluation of stool consistency and presence of blood in stools. Tissue myeloperoxidase (MPO) activity was carried out to measure neutrophil accumulation. Isolated distal portions of colon were placed in an embedding frame, preserved in 10% formalin, sliced, mounted and stained with hemotoxylin & eosin and then histologically scored. Oral administration of *T. terrestris* (40, 80 and 160 mg/kg) extract revealed a markedly reduced disease severity, attributed to reductions in occult blood, gross bleeding, and diarrhea. The colon length and colon weight of the animals treated with *T. terrestris* extract was found to be comparable to vehicle control. MPO activity in *T. terrestris* extract treated groups was found to be decreased as compared to the disease control group. Administration of *T. terrestris* extract significantly reduced the histological injury induced by DSS**.** The inflammation score and crypt damage score was found to be significantly improved in *T terrestris* treated DSS mice. The results showed that *T. terrestris* extract has a potential to suppress colitis in mice as indicated by the macroscopic, microscopic and biochemical evaluations. The findings of this study elucidate the anti-IBD and anti-inflammatory properties of *T. terrestris*.

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INTRODUCTION

Inflammatory bowel disease (IBD) refers to two related but different diseases namely ulcerative colitis and Crohn's disease. Ulcerative colitis (UC) and Crohn's disease is chronic inflammatory bowel diseases (IBD) in which the role of interactions between genetic, immunologic, microbial and environmental factors is expected, but exact etiology and pathogenesis still remain unclear (Hock et al, 2011). Crohn's disease is the chronic inflammation of the intestine that is usually confined to the ileum, the terminal portion of the small intestine. Ulcerative colitis is a similar inflammation of the colon, or large intestine. The general morbidity and weight loss in individuals with IBD can be attributed to leukocyte sequestration in the gut. The balance between pro and anti-inflammatory cytokines secreted by T cells regulates both the initiation and perpetuation of these diseases. IBD is related with multiple etiologic theories such as genetics, immunology and environment (Alsted, 2001 and Kirsner et al, 1982). In recent years, a study of animal models of IBD pathogenesis has shown that immune imbalance led to the inflammation in the gastrointestinal tract (Strober et al, 2002). It has been hypothesized that the chronic inflammation in IBD is due to a disturbed balance of pro and anti-inflammatory cytokines. IBD has demonstrated an increased expression of pro-inflammatory cytokines, chemokines, and adhesion molecules such as tumor necrosis factor-α (TNF-α), interleukin (IL-1, IL-2, IL-6, IL-8) interferon-γ (IFN-γ), intercellular adhesion molecule 1, vascular adhesion molecule 1, and E selection. Proinflammatory cytokines (TNF-α and IL-1 α) are considered as pivotal mediators in inflammatory conditions like rheumatoid arthritis, inflammatory bowel diseases, and psoriasis. Elevated TNF-α synthesis has been associated with the development of diabetes, septic

shock, tumorigenesis, rheumatoid arthritis, psoriatic arthritis and inflammatory bowel disease. Many biologic therapies are being evaluated for the treatment of chronic inflammatory bowel diseases (Sandborn et al, 2002, Sander van der Marel et al, 2002 and Sing et al, 2001). However, during recent years, the mainstay therapies for IBD have been anti-inflammatory drugs and glucocorticosteroids (GCS) (Xu and Pan, 1999; Jani and Rgueiro, 2002).

T. terrestris, has been traditionally, used for gastrointestinal diseases. It has been used in Ayurvedic medicine to treat a variety of diseases for several thousand years (Rao, 2002). Currently, tumor necrosis factor-α (TNF-α) inhibitors from natural origins are being advanced for the treatment of inflammatory disorders. *T. terrestris* has been found to contain chemical constituents that inhibit TNF-α and IL-1 α production in various in vitro models (Ashraf, 2003). Presently, there is no evidence whether IBD patients can benefit from *T. terrestris,* as well as any animal model experiments done.

A number of animal models mimicking different aspects of IBD were developed. In mice, an experimental colitis is probably the most commonly induced by oral administration of dextran sodium sulfate (DSS)(Okayasu et al, 1990). DSS induces acute colitis with symptomatic and histopathologic findings such as rectal bleeding, body weight loss, shortening of the colon, distortion of crypts, loss of goblet cells and infiltration of leukocytes. It is accompanied by increased transcription levels of inducible forms of cyclooxygenase (COX-2) and NO synthase (iNOS), and proinflammatory cytokines including tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) (Gravaghi et al, 2011 and Yan et al, 2009). The purpose of the present investigation is to evaluate the effectiveness of *T. terrestris* against dextran sulphate induced colitis in mice.

Commercially available extract of *T. terrestris* was obtained from Natural Remedies Private Ltd., Ashwathilakhsmi Mansion, N° 364, Jayanagar, Bangalore, India. The company manufactures the extract routinely to be used in various formulations used for various ailments. The details are as follows:

Name: *Tribulus terrestris* Extract
Code: NR-TTE-40
Batch N°: TT/05004
Net weight: 1 kg

Storage conditions: Stored in an air-tight container at room temperature

MATERIALS AND METHODS

Chemicals

Dextran Sodium Sulphate (DSS) (molecular weight: 40,000) was obtained from ICN Biomedicals (Ohio, USA). Sulfasalazine was purchased from Sigma Co., St. Louis, MO, U.S.A. All other chemicals and solvents used were of analytical grade.

Animals

Male BALB/c mice obtained from the Animal Facility, Department of Toxicology, Orchid Chemicals & Pharmaceuticals Ltd., Chennai, India were used in this experiment. The mice were aged 8 to 9 weeks at the initiation of the study. The animals were kept at controlled environmental conditions where the temperature of the experimental room was maintained at 20 - 23°C, relative humidity at 50 – 60% with a 12-h light/dark cycle. All animals were given unlimited access to a standard pellet diet and water. The animals were housed individually in standard mice cages and were acclimatized for 7 days before initiation of the treatment.

Six groups of mice, comprising 8 males per group were randomly selected for the study. Group one (G1) served as untreated control, group 2 (G2) served as the chemical (DSS) induced group, group 3 (G3) served as positive control (treated with sulfasalazine) and the remaining groups 4, 5 and 6 (G4, G5 and G6) were treated with *T. terrestris* extract.

Induction of Colitis

Colitis was induced in animals of groups G2 to G6 by adding Dextran sulfate sodium (DSS - mol. wt. 40,000) to the drinking water from day 1 to day 7 (Soriano et al, 2000; Taniguchi et al, 1998; Umene, et al, 1994; Wu et al, 1998) *ad libitum*. Animals of group G1 received distilled water alone.

Treatment

T. terrestris extract was dissolved in distilled water and administered to groups G4, G5 and G6 at the dose levels of 40, 80 and 160 mg/kg body weight respectively. The positive control group (G2) received sulfasalazine at the dose level of 100 mg/kg b.wt. Vehicle control group (G1) animals received distilled water alone. The administration was carried out from day 1 to day 7 by oral gavage. Dose volume was maintained at 10 ml/kg body weight for all the groups. The daily dose of individual animal was adjusted based on the recent body weight.

Clinical Disease Activity Measurements

A daily clinical assessment was performed in all the animals, including body weight, evaluation of stool consistency, and presence of blood in stools. The clinical score was determined as per the following procedure (Hartmann et al, 2000).

Parameter	Change	Score
Weight Loss	0	0
	1 to <5%	1
	5 to <10%	2
	10 to <20%	3
	>20%	4
Stool Consistency	Normal *	0
	Loose	2
	Diarrhea	4
Fecal Occult Blood	Normal #	0
	Positive	2
	Gross Bleeding	4

Key: Normal *: well-formed stool pellets, Loose: pasty stools that do not stick to the anus; Diarrhea: liquid stools that stick to the anus, Normal #: absence of occult blood in stool, Positive: presence of occult blood in stool, and Gross Bleeding: profuse bleeding from the rectum.

Body weight, rectal bleeding, and stool consistency along with clinical signs were observed at the start of the experiment and daily thereafter. Disease activity index

(DAI) was measured as the combined scores of 1) weight loss, 2) stool consistency, and 3) bleeding divided by 3 (Kim and Berstad, 1992; Murthy et al, 1993).

The mice were sacrificed 24 h after the last treatment (Day 7) by an overdose of CO_2. The colons were dissected and the length and weight were measured. Segments of colon were used for the measurement of tissue myeloperoxidase activity. Histopathology of colon was also carried out.

Assay for myeloperoxidase activity

Tissue myeloperoxidase (MPO) activity was carried out to measure neutrophil accumulation (Krawisz et al, 1984). Segments of the colon were collected with a 4 - 8 mm biopsy punch, opened by a longitudinal incision, gently rinsed with ice-cold saline, weighed, and re-suspended in 50 mM phosphate buffer containing hexadecyl trimethyl ammonium bromide (HTAB; 5 mg/ml) at a ratio of 2 ml HTAB buffer per 50 mg tissue. Colonic segments were cooled in ice, homogenized, sonicated for 30 seconds, and freeze-thawed three times. The resulting lysate was centrifuged at 13000 *g* for five minutes at 4°C. Aliquots were transferred to microtitre plates and MPO activity was assayed in HTAB buffer containing hydrogen peroxide and *o*-dianisidine. The colorimetric response was monitored at the optical density of 460 nm and the reaction was stopped with 0.1% sodium azide.

The crypt was scored at grades 0 to 4 as follows:

Grade of Disease	Score
Crypt intact	0
Loss 1/3 crypt	1
Loss of 2/3 crypt	2
Loss of entire crypt with intact surface epithelium	3
Loss of entire crypt with erosion of surface epithelium	4

These changes were quantified as to the percentage involvement by the disease process as follows:

Extent of Damage (% involvement)	Score
1-25%	1
26-50%	2
51-75%	3
76-100%	4

Crypt damage score was determined as the sum of the grade of the crypt and percent area score.

The inflammation was evaluated subjectively on a 0 to 3 grade as follows:

Score for Severity Score	Score
Normal	0
Focal inflammatory cell infiltrate	1
Inflammatory cell infiltrate, gland drop out and crypt abscess	2
Mucosal ulceration	3

The extent of involvement was estimated as to, of the total surface area as follows:

Extent of Damage (% involvement)	Score
1-25%	1
26-50%	2
51-75%	3
76-100%	4

The inflammation score was determined as the sum of the inflammation grade and the percent extent score.

Histopathological evaluation of colon damage

Isolated distal portions (5 cm segment) of colon were placed in an embedding frame and preserved in 10% formalin. Subsequently, the colon was sliced, mounted and stained with hemotoxylin & eosin and then scored. Histological damage was scored as described by H.S. Cooper (1993).

Statistical Analyses

The data were expressed as mean±S.E.M. The data were checked for homogeneity using Bartlett's test. Statistical significance of differences between treatment and disease control groups were determined. All homogenous data were analyzed by ANOVA followed by Dunnett's test. All heterogenous data were analyzed by F-test followed by Student's t-test. Differences were considered statistically significant for $p \leq 0.05$ and 0.01. Percent inhibitory effects were calculated using the following formula:

Percent inhibition (%) = disease control – treated / disease control x 100

RESULTS

DSS induced colitic mice after administration of the extract were monitored daily for clinical signs for 7 days. All animals survived until sacrifice on day 7. Clinically, hematochezia and diarrhea were observed after administration of 2.0% DSS.

Following induction of colitis, the body weight was significantly decreased in the DSS treated group on day 7 of DSS treatment as compared to the vehicle control group (Table 2 and Figure 2). On day 7, a significant ($P \leq 0.01$) decrease in mean body weight, (18.2±0.86) in the DSS group was observed as compared to the control group (25.3±1.14). On day 7, the mean body weight (Table 2 and Figure 2), of animals treated with *T. terrestris* extract at the dose levels of 40, 80 and 160 mg/kg body weights were 20.7±1.02, 21.7±0.54 and 23.8±0.66 grams respectively. This was significantly different from the mean body weight of the control group as well as significantly higher as compared to the mean body weight recorded in the DSS group. Similarly the mean body weight of animals treated with sulfasalazine was significantly increased (20.9±1.01) as compared to the DSS induced group (18.2±0.86). On day 7, the percent body weight loss (Table 3 and Figure 3) in DSS group was -35.8 %, sulfasalazine group -17.5 % and *T. terrestris* extract treated groups at the dose levels of 40, 80 and 160 mg/kg b.wt. were -20.8, -13.2 and -2.9 % respectively.

Oral administration of *T. terrestris* (40, 80 and 160 mg/kg) and sulfasalazine treated groups (100 mg/kg) revealed a markedly reduced disease severity (Table 4 and Figure 9). The reduction in disease severity was attributed to reductions in occult blood, gross bleeding, and diarrhea. Occult blood and diarrhea was observed from day 3 onwards in the animals treated with DSS alone. A progressive increase in the overall DAI was observed in the disease control group animals (DSS-treated) as compared to the vehicle control animals from day 3 onwards. On day 7, the observed increase of DAI (4.0±0.0) in the disease control group was found to be markedly greater than those observed in the *T. terrestris*

Table 1: Treatment with different oral doses of T. terrestris extract (40, 80 and 160 mg/kg) on clinical score in DSS-induced colitis

Days of Treatment	Mean±S.E.M. (n = 8)					
	Group1 (Control)	Group2 (DSS 2%)	Group 3 (Sulfasalazine @ 100mg/kg)	Group 4 (*T. terrestris* @ 40 mg/kg)	Group 5 (*T. terrestris* @ 80 mg/kg)	Group 6 (*T. terrestris* @ 160 mg/kg)
Day 0	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00
Day 1	0.0±0.00	1.0±0.00	0.1±0.13	0.1±0.08	0.6±0.15	0.0±0.00
Day 2	0.0±0.00	2.0±0.00	0.8±0.09	0.8±0.09	1.1±0.06	0.8±0.13
Day 3	0.0±0.00	2.8±0.16	0.9±0.06	1.6±0.15	1.3±0.16	0.9±0.11
Day 4	0.0±0.00	3.4±0.18	1.8±0.09	1.9±0.18	1.6±0.15	1.7±0.19
Day 5	0.0±0.00	4.0±0.00	2.1±0.20	2.2±0.16	2.1±0.18	1.8±0.13
Day 6	0.0±0.00	4.0±0.00	2.1±0.22	2.6±0.21	2.1±0.21	1.8±0.13
Day 7	0.0±0.00	4.0±0.00	2.1±0.13	2.8±0.16	2.1±0.23	1.8±0.16

Table 2: Treatment with different oral doses of *T. terrestris* extract (40, 80 and 160 mg/kg) on mean body weight (g) in DSS-induced colitis

Days of Treatment	Mean±S.E.M. (n = 8)					
	Group1 (Control)	Group2 (DSS 2%)	Group 3 (Sulfasalazine @ 100mg/kg)	Group 4 (*T. terrestris* @ 40 mg/kg)	Group 5 (*T. terrestris* @ 80 mg/kg)	Group 6 (*T. terrestris* @ 160 mg/kg)
Day 0	24.5±1.10	25.3±1.22	24.0±1.22	24.0±0.92	23.9±0.98	23.7±1.00
Day 1	24.4±1.07	24.9±1.20	22.9±1.20	23.5±1.01	22.7±0.76	23.0±0.72
Day 2	24.5±1.06	24.4±1.04	22.2±1.07	22.1±0.99	21.6±0.72 ↓	23.0±0.74
Day 3	24.7±1.09	21.6±1.09	21.9±1.09	22.0±1.08	21.7±0.72 ↓	23.0±0.75
Day 4	24.8±1.08	21.1±1.05 ↓	21.7±1.06	22.0±1.05	21.6±0.65 ↓	23.1±0.72
Day 5	25.0±1.08	21.0±1.15 ↓	21.6±0.93 ↓	21.8±1.02 ↓	21.5±0.72 ↓	23.2±0.71
Day 6	25.1±1.12	19.7±0.94 ↓↓	21.4±0.97 ↓	21.3±0.98 ↓↓	21.4±0.56 ↓↓	23.5±0.69
Day 7	25.3±1.14	18.2±0.86 ↓↓	20.9±1.01 ↓	20.7±1.02 ↓↓	21.7±0.54 ↓	23.8±0.66

Key: ↓- Significantly lower than control (P≤0.05), ↓↓- significantly lower than control (p< 0.01), N = 8/group

Table 3: Treatment with different oral doses of T. terrestris extract (40, 80 & 160 mg/kg) on mean body weight loss (g) in DSS-induced colitis

Days of Treatment	Mean±S.E.M. (n = 8)					
	Group1 (Control)	Group2 (DSS 2%)	Group 3 (Sulfasalazine @ 100mg/kg)	Group 4 (*T. terrestris* @ 40 mg/kg)	Group 5 (*T. terrestris* @ 80 mg/kg)	Group 6 (*T. terrestris* @ 160 mg/kg)
Day 0	0±0	2.8±1.15	-2.6±1.52	-2±2.33	-2.5±0.83	-3.4±2.56
Day 1	-0.3±0.65	1.4±1.19	-7.5±1.55	-4.7±3.04	-7.7±2.16	-6.6±3.48
Day 2	0.1±0.47	-0.6±1.24	-10.4±1.05	-12.1±5.85	-13.9±5.42	-6.4±3.55
Day 3	0.9±0.41	-13.6±1.45	-12.3±1.12	-12.5±5.79	-13.6±5.43	-6.7±3.93
Day 4	1.4±0.38	-16.4±1.9	-12.9±1.16	-12.5±5.68	-14±5.33	-6.2±3.76
Day 5	1.9±0.49	-17.2±2.34	-13.4±1.68	-13.4±5.75	-14.5±5.42	-5.7±3.92
Day 6	2.3±0.54	-25.2±4.6	-14.5±1.3	-16.5±6.65	-14.9±5.03	-4.5±3.81
Day 7	3.1±0.54	-35.8±4.6	-17.5±1.3	-20.8±6.65	-13.2±5.03	-2.9±3.81

Table 4: Treatment with different oral doses *T. terrestris* extract (40, 80 and 160 mg/kg) on disease activity index (calculated score) in DSS-induced colitis

Days of Treatment	Mean±S.E.M. (n = 8)					
	Group1 (Control)	Group2 (DSS 2%)	Group 3 (Sulfasalazine @ 100mg/kg)	Group 4 (*T. terrestris* @ 40 mg/kg)	Group 5 (*T. terrestris* @ 80 mg/kg)	Group 6 (*T. terrestris* @ 160 mg/kg)
Day 0	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00
Day 1	0.0±0.00	2.0±1.00	1.1±0.94	1.1±0.94	1.3±0.72	0.5±0.50
Day 2	0.0±0.00	2.5±0.5	1.4±0.59	1.4±0.59	1.5±0.47	0.9±0.13
Day 3	0.0±0.00	3.4±0.63	1.5±0.53	1.8±0.22	1.7±0.34	1.5±0.53
Day 4	0.0±0.00	3.7±0.31	1.9±0.13	2±0.03	1.8±0.22	1.8±0.16
Day 5	0.0±0.00	4.0±0.00	2.0±0.03	2.1±0.09	2.1±0.06	1.9±0.09
Day 6	0.0±0.00	4.0±0.00	2.0±0.03	2.8±0.19	2.1±0.06	1.9±0.09
Day 7	0.0±0.00	4.0±0.00	2.1±0.06	2.9±0.13	2.1±0.06	1.9±0.13

Table 5: Treatment with different oral doses of *T. terrestris* extract (40, 80 and 160 mg/kg) on colon length (cm), colon weight (g), MPO activity, inflammation and crypt score in DSS-induced colitis

Parameters	Mean±S.E.M. (n = 8)					
	Group1 (Control)	Group2 (DSS 2%)	Group 3 (Sulfasalazine @ 100mg/kg)	Group 4 (*T. terrestris* @ 40 mg/kg)	Group 5 (*T. terrestris* @ 80 mg/kg)	Group 6 (*T. terrestris* @ 160 mg/kg)
Colon Length (cm)	10.6 ±0.36	7.1 ±0.21	9.1 ±0.30	8.4 ±0.14 ↓↓	9.1 ±0.18 ↓↓	10.8±0.19
Colon Weight (g)	290.4±2.62	225 ±4.94	287.3±3.87	260.5±10.97 ↑	283.5±4.90	300±6.68
Myelo-peroxidase *	0.0 ±0.00	1600.3 ±14.70	330.6 ±11.10 ↓↓	1059.5 ±9.16 ↓↓	714.9 ±19.50 ↓↓	287.1 ±25.00 ↓↓
Inflammation Score	0.5 ±0.33	7.3 ±0.37	1.0 ±0.38	6.8 ±0.33 ↑↑	3.8 ±0.59 ↑↑	1.0 ±0.38
Crypt Score	0.3 ±0.25	6.3 ±0.33	0.6 ±0.26	5.8 ±0.33 ↑↑	3.5 ±.82 ↑↑	0.8 ±0.31

Key: ↓↓- significantly lower than control (p ≤ 0.01), ↑ - Significantly higher than control (P≤0.05), ↑↑ - Significantly higher than control (p ≤ 0.01), * = significance compared to disease control

Table 6: Summary of the effect of *T. terrestris* extract in DSS-induced colitis at different dose levels (40, 80 and 160 mg/kg)

Parameters	Inhibition (%)			
	Group 3 (Sulfasalazine @ 100mg/kg)	Group 4 (*T. terrestris* @ 40 mg/kg)	Group 5 (*T. terrestris* @ 80 mg/kg)	Group 6 (*T. terrestris* @ 160 mg/kg)
Clinical Score	53	43	49	59
Disease Activity Index	44	34	41	51
Colon Length	22	15	22	34
Colon Weight	20	8	19	24
Inflammation Score	86	7	48	86
Crypt Score	91	21	52	90
Myeloperoxidase Activity	79	34	55	82
Overall Activity	57	20	40	61

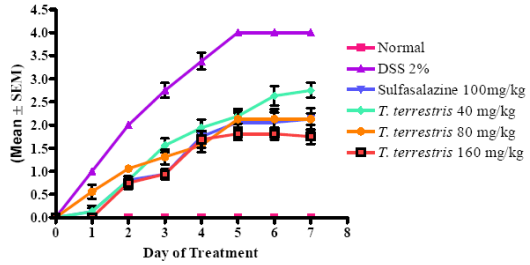


Fig. 1: Effect of *T. terrestris* extract on clinical score in mice

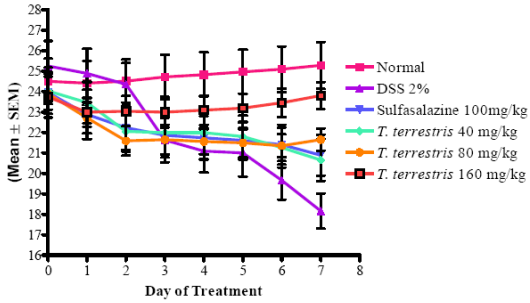


Fig. 2: Effect of *T. terrestris* Extract on body weight (g) in mice

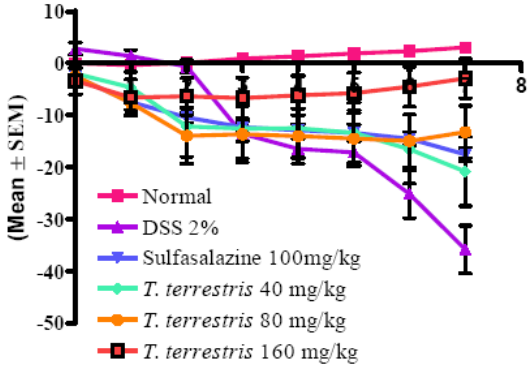


Fig. 3: Effect of *T. terrestris* extract on percent weight loss/gain (%) in mice

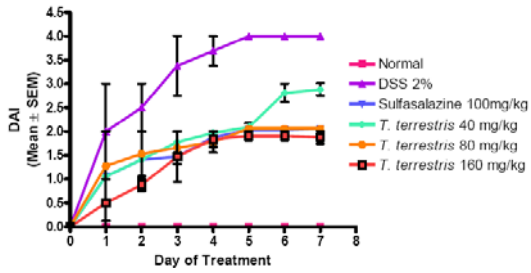


Fig. 4: Effect of *T. terrestris* extract on disease activity Index in mice

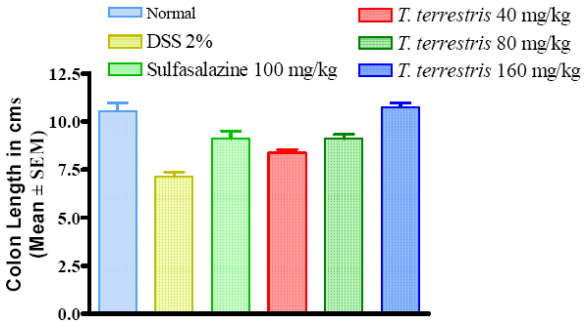


Fig. 5: Effect of *T. terrestris* extract in preventing DSS-induced colon length

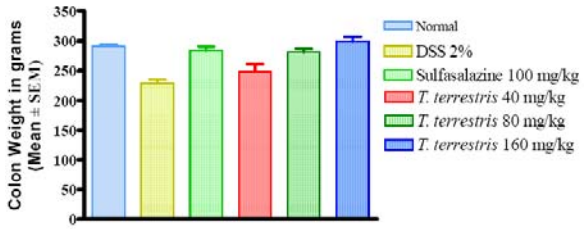


Fig. 6: Effect of *T. terrestris* extract in preventing DSS-induced colon weight

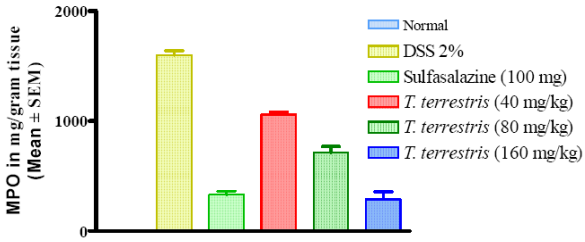


Fig. 7: Effect of *T. terrestris* extract on myeloperoxidase in mice

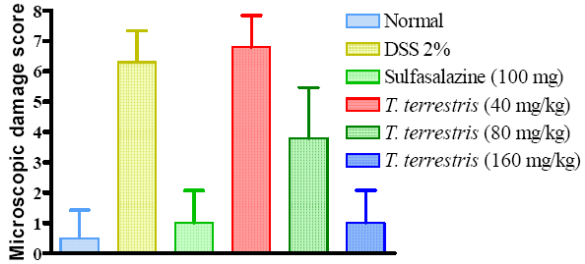


Fig. 8: Effect of *T. terrestris* extract in reducing inflammation damage score

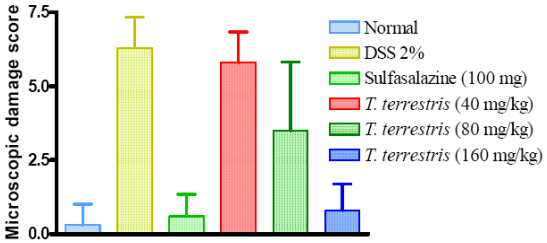


Fig. 9: Effect of *T. terrestris* extract in reducing crypt damage score

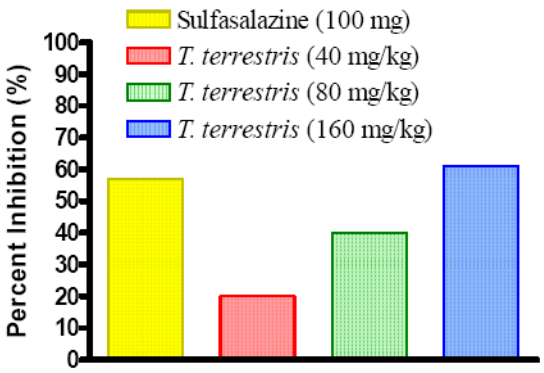
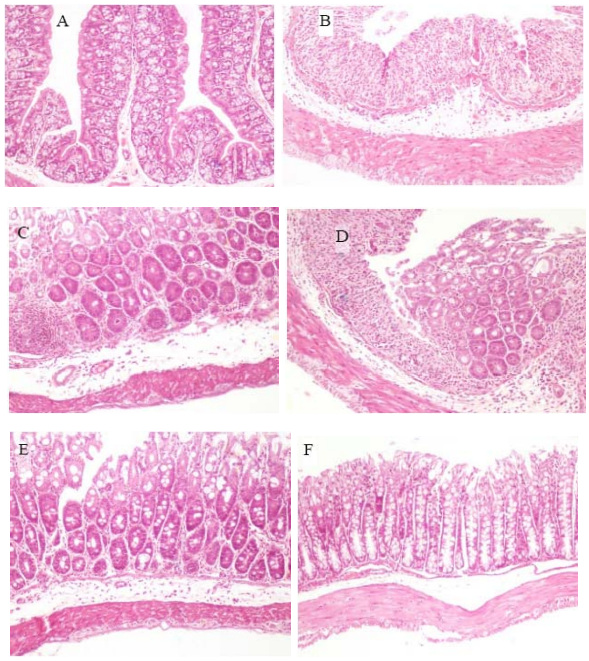


Fig. 10: Dose dependent effect (overall) of *T. terrestris* in DSS-induced colitis



Hematoxylin and Eosin (H & E) Stained Sections of Colon. Magnification 40x for all photomicrograph.

Figure N°:11. A: Negative control mouse with normal histomorphology; B: Animal treated with DSS at 2%; C: Animal treated with Sulfasalazine at 100 mg/kg; D: Animal treated with *T. terrestris* at 40 mg/kg; E: Animal treated with *T. terrestris* at 80 mg/kg; F: Animal treated with *T. terrestris* at 160 mg/kg

extract treated animals at the dose levels of 40, 80 and 160 mg/kg body weight (2.9±0.13, 2.1±0.06 and 1.9±0.13) as well as animals treated with sulfasalazine at the dose levels of 100 mg/kg body weight (2.1±0.06).

Decreased colon length (Table 5) observed in the disease control group mice, which was significantly ($P<0.01$) lower (7.1±0.21) as compared to the vehicle control group. Mean colon length in vehicle control group was 10.6±0.36 cm (measured from rectum to lower part of ceacum). The increase in colon length (Table 5) observed in DSS induced colitic animals treated with sulfasalazine at the dose level of 100 mg/kg body weight was 9.1±0.30 and animals treated with *T. terrestris* at the dose levels of 40, 80 and 160 mg/kg body weight were 8.4±0.14, 9.1±0.18 and 10.8±0.19 cm respectively (Figure 5).

Evaluation of colon weight revealed a dose dependent increase in DSS induced colitic animals treated with *T. terrestris,* namely 260.5±10.97, 283.5±4.90 and 300.0±6.68 grams at the dose levels of 40, 80 and 160 mg/kg body weight respectively. In case of vehicle control, disease control group and sulfasalazine groups colon weights were found to be 290.4±2.62, 225.0±4.94 and 287.3±3.87 grams respectively (Table 5 and Figure 6).

The results of Myeloperoxidase (MPO) activity correlated closely with other parameters studied *viz.*, clinical, macroscopic, and histological grading of inflammation in the experimental groups. MPO activity in the intestinal tissue of the disease control group (1600.3±14.70) was significantly increased as compared to the vehicle control group (-281.1±4.44) (Figure 7). In contrast, MPO activity in DSS plus (Table 5 and Figure 7) sulfasalazine (100 mg/kg b.wt.) or DSS plus *T. terrestris* treated groups at the dose levels of 40, 80 and 160 mg/kg b.wt., decreased by 79 and 33, 55 and 82 % as compared with that of the disease control group respectively.

The histological sections obtained from the disease control group on day 7 revealed lesions of the distal colon consisting of multifocal areas of mucosal erosion (Figure 11). The intestinal sections displayed a loss of epithelial cells, reduction in goblet cells, shortening, dilation and collapse of crypts, submucosal edema, and transmural distribution of mixed inflammatory infiltrates consisting of macrophages, lymphocytes, and neutrophils Figure 11B). None of the mice in the control group developed any histologic signs of colitis (Figure 11A). Administration of sulfasalazine (100 mg/kg b.wt., Figure 11C) or DSS plus *T. terrestris* extract groups (40, 80 and 160 mg/kg b.wt., Figures 11D, 11E & 11F) significantly reduced the histological injury induced by DSS**.**

The inflammation score (Table 5 and Figure 8) and crypt damage score (Table 5 and Figure 9) was found to be significantly improved in *T terrestris* extract treated DSS mice. Inflammation score was found to be significantly increased in the disease control group (7.3±0.37 versus vehicle control 0.5±0.33) and as reduced by *T. terrestris* (6.8±0.33, 3.8±0.59 and 1.0±0.38) administered (Table 9) at the dose levels of 40, 80 and 160 mg/kg body weight respectively.

DSS also induced significant crypt damage (6.3±0.33 versus control – 0.3±0.25). Crypt damage was reduced by *T. terrestris* extract (5.8±0.33, 3.5±0.82 and 0.8±0.31) respectively. The inflammation and crypt damage score was significantly improved in sulfasalazine-treated DSS mice also (0.6±0.26).

DISCUSSION

Inflammatory bowel disease is a major illness of the gastrointestinal tract, the exact pathogenesis of which is unknown. The initial mucosal insult leads to production and release of pro-inflammatory cytokines with attraction of more immune cells and disruption of mucosal integrity. Exposure of specific epitopes on diseased bowel mucosa through toxic, infectious, or immune-mediate effects is a possible mechanism in the pathogenesis of IBD (Podolsky, 2003). The immune response is the result of a balance between Th1 and Th2 subtype responses (Adorini and Sinigaglia, 1999). The Th1-type response is involved in the pathogenesis of several autoimmune and chronic inflammatory disorders such as Crohn's colitis (Mizoguchi et al, 1996). It was recently shown that, both in animals and humans, the anti-inflammatory effects of Th1-mediated cytokines, alleviate the disease (Madsen et al, 1997). Thus IBD can be perceived as an imbalance between pro-inflammatory and anti-inflammatory cytokines. Acute DSS-induced colitis is very similar to that observed in human inflammatory conditions of IBD (Plevy et al, 1997). The histological aspects of intestinal damage in DSS induced colitis share many similarities with those seen in patients affected by IBD and, in particular ulcerative colitis (Okayasu et al, 1990 and Cooper et al, 1993). DSS-induced colitis model in mice is similar to human ulcerative colitis in terms of histological features. It affects the colon portion and induces non-transmural inflammation, massive necrosis of mucosal and submucosal layers, mucosal edema, neutrophil infiltration of the mucosa and submucosal ulceration.

Various types of inflammatory cells play important roles in the modulation of intestinal immunity and inflammation (Nakamura et al, 1993; Nakamur et al, 2006 and Oshitani et al, 1995). The mice model of DSS induced colitis was used because it is a well-characterized model with a predictable disease progression, and also has numerous clinical, biochemical, and histological features that closely resemble human IBD. The major finding of this study is that *T. terrestris* extract can exert effects on established DSS induced colitis. DSS and *T. terrestris* extract was administered from day 1 onwards. Biochemical or histological data revealed that the mice receiving *T. terrestris* extract had markedly decreased the clinical signs of colitis, as determined by the disease activity index. The significant correlation between the disease activity index and other markers of disease activity such as the MPO activity and tissue damage score further support this observation.

MPO has been used as an indicator of neutrophil influx in several experimental models for colitis (Hogaboam et al, 1995 and Rachmilewitz et al, 2002). Significantly elevated colonic MPO activity in disease control group mice was observed. Neutrophils have been reported to play a central role in active colitis (Nakamura et al, 1993 and Oshitani et al, 1995), and DSS-induced colitis was manifested by neutrophil infiltration into the colon (Okayasu et al, 1990; Cooper et al, 1993 and Gaudio et al, 1999). The colonic MPO was reduced in colitic mice orally treated with *T. terrestris* extract. The suppressive effects of *T. terrestris* on the development of DSS-induced experimental colitis, since tissue MPO activity was significantly attenuated in the *T. terrestris* treated mice as compared to the vehicle control mice. MPO activity is an index of neutrophil infiltration, and the suppression of MPO activity indicates that *T. terrestris* blocked the infiltration of neutrophils into the colonic mucosa. Neutrophil-derived MPO may contribute to tissue damage by virtue of its ability to oxidize, chlorinate, and nitrosylate proteins in the presence of neutrophil-derived oxidants and nitric oxide. The production of reactive oxygen metabolites and MPO by the excess infiltration of neutrophils directly induces tissue injury (Krawisz et al, 1984). Thus, it appears that the inhibition of neutrophil infiltration into the colonic mucosa by *T. terrestris* suppressed the inflammatory responses, which lead to the development of DSS-induced colitis.

The present study showed that treatment with *T. terrestris* extract suppressed the development of DSS-induced colitis as assessed clinically (improvement in weight loss, physical activity, bloody diarrhea, shortening of the colon, and peri-anal erosions) by 20, 40 and 61 % when treated at the dose levels of 40, 80 and 160 mg/kg body weight respectively. Interestingly, the results obtained from *T. terrestris* at the dose level of 160 mg/kg body weight was comparable to the results obtained from Sulfasalazine treated group (100 mg/kg body weight) which showed significant protection (57 %) against DSS-induced colitis proved by biochemical, macroscopic and microscopic examinations.

The results presented in the current study demonstrated a protective effect for *T. terrestris* extract in DSS-induced colitis. The results showed that *T. terrestris* extract has a potential to suppress colitis in mice as indicated by the macroscopic, microscopic and biochemical evaluations. The performed biochemical assays indicated that administration of *T. terrestris* extract reduces MPO activity. The findings of this study elucidate the anti-IBD and anti-inflammatory properties of *T. terrestris* extract.

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