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Protective effect of *Abies pindrow* on dextran sulphate sodium induced ulcerative colitis in rats

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The present study was designed to evaluate the protective effect of the leaves of *Abies pindrow* in dextran sulphate sodium (DSS) induced ulcerative colitis in rats in an attempt to search for a safe, effective, and economic treatment of ulcerative colitis. Petroleum ether extract of leaves of *A. pindrow* (PEEAP)was used and its phytochemical screening was performed. Dextran sulphate sodium induced ulcerative colitis model was used for the induction of disease. Rats were divided into six groups (n=6) namely, vehicle control, disease control, standard group (Sulphasalazine – 50mg/kg) and test group (PEEAP – 100, 200 & 400 mg/kg). Disease activity index (DAI) was recorded daily from day 6 onward. After 11 days, animals were sacrificed and evaluated for colon mucosal damage index (CMDI) and the level of myeloperoxidase activity (MPO), malondialdehyde activity (MDA), Super oxide dismutase (SOD), catalase in the homogenized colon tissue. The animal treated with PEEAP (400 mg/kg) showed significant higher colon length than the disease control group. CMDI were found to be significantly lower in the treatment group as compared to the disease group. DAI was found to be significantly lower in the treatment group as compared to the diseased control group. It also showed a lower level of MPO and MDA and a higher level of SOD and catalase than those observed in the disease group. PEEAPsignificantly reduced the severity of the ulcerative colitis produced by DSS. The anti-inflammatory, anti-ulcer and antioxidant activity of this plant might be responsible for its protective role in ulcerative colitis. Further studies are suggested to isolate the active principle responsible for the activity and necessary to confirm the exact mechanism of action.

Keywords: *Abies pindrow*, Anti-inflammatory action, Antioxidant action, DSS, Ulcerative colitis.

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Introduction

Inflammatory bowel disease (IBD) represents a group of idiopathic chronic inflammatory condition of the gastrointestinal tract. Inflammatory bowel disease describes two major chronic conditions like Crohn's disease and ulcerative colitis (UC), both having different pathological features[1].

Clinically UC is related to the inflammatory and ulcerative condition of the colon and rectum. UC is typically related to the innermost layer of mucosa, whereas CD is a segmental, transmural disorder involving any part of the gastrointestinal tract[2]. The pathophysiology of ulcerative colitis is not completely known, but increasing evidence suggests that the condition may be caused by dysfunction of the intestinal immune system leading to the dysfunctional intestine mucosa against enteric bacteria in a genetically susceptible host[3]. The aetiology of the disease is incompletely understood but it mostly related to genetic factor and environmental triggers or modifiers[4]. Although the incidence and prevalence of inflammatory bowel disease are mostly related to Europe and North America, they continue to rise in the low-incidence areas such as southern Europe, Asia and much of the developing world. In developing countries, UC emerged first and then CD followed[5].

In India, UC was first reported in 1964 and CD in 1986. During the last 10 years, CD is more frequently reported in India especially southern India. In India, there is a report of UC/CD ratio of 8:1[(ref 6)].

Because of no specific pathogen to mediate the known effect, a great deal of attention has been focused on immune deregulation. Accordingly, many different animal models with defined knockouts of inflammatory factors such as IL-2, IL-10, and T cell receptor, as well as seemingly unrelated molecules, such as multidrug resistance protein appears to result in bowel inflammation[7]. Lymphocytes, cytokines, and adhesion molecules were immunologically dysregulated and have been targeted for therapeutic intervention[8].

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Management of ulcerative colitis involves mainly the use of anti-inflammatory agents (aminosalisylates) and immunosuppressive agents like steroids (corticosteroids) and antibiotics. Long term use of glucocorticoids was related to a high rate of relapse and undesirable toxicity. On the other hand immunosuppressants (azathioprin and 6-mercaptopurine) was effective in maintaining this condition, but many patients were intolerant or resistant to thiopurine. Novel research developed monoclonal antibodies against TNF-α, which gave prominent activity against ulcerative colitis.[7]But these were highly expensive and having side effects. Consequently, there is a need for an alternative, more effective, cost beneficial agent for the management of the disease.

During the past decade, the herbal system of medicine has become a topic of global importance. Herbal medicines are approved by WHO as an essential component of primary health care, especially in developing countries like India.[9]

The use of plant or their extracts for the treatment of human disease predated the earliest stages of recorded civilization, dating back at least to the Neanderthal period. By the 16th century, the botanical garden provided a wealth of *Materia medica* for teaching therapeutic use and herbal medicine flourished until the 17th century when more scientific 'pharmacological' remedies were discovered[10]. Herbal treatment was considered an effective treatment and a good source of secondary metabolites. Till today various scientists evaluated possible medicinal plants therapeutic uses and safety from different plant species. Assurance of the safety, quality and efficacy of herbal plants has now become an important issue in developing countries.

Concurrently, many people in developed countries have begun to use herbal treatment as an alternative or complementary therapy. *Abies pindrow*, belonging to the family Pinaceae is an important ayurvedic medicinal herb. *Abies pindrow* is commonly known as "talisaptra" and "West Himalayan fir/silver fir". Botanical name: *Abies pindrow*, Synonyms: *Abies himalayensis, Pinus pindrow, Pinus spectabilis* var. *pindrow*, Plant family: Pinaceae, Kingdom: Plantae, Division: Pinophyta, Class: Pinopsida, Genus: Abies, Vernacular name: In Sanskrit: *Talisaptra, Talisa, Granthiparna*, In Hindi: *Morinda, Dodimma, Jhilla, Tosh, Rei, Rai, Span*, In Pakistan: *Partal* or *Palundar*, Unani: *Zarnab*. In *Ayurveda*, it is used as a remedy for fever, respiratory, and inflammatory ailments. Anti-diabetic, anti-inflammatory, analgesic, hypnotic, and anti-ulcer activities in rats, hypotensive effect in dogs, and endurance enhancing in swim stress in mice have been reported for extracts and fractions from *A. pindrow*leaves.[11,12]Leaves mainly contain pentacyclic triterpenoid (pindralactone), flavonoid (chalcones), carbohydrates, fatty acid, pinitol and heterocyclic complex (maltol)[11,13]. Methanolic extract of *A. pindrow* aerial parts showed antidepressant and antistress activity in animal models[14]. Various Crude extracts of *A. pindrow* aerial parts showed antianxiety activity in an animal model[15].

Anti-inflammatory activity and suppression of NF-κB by pinitol (glycoside) have been reported and anti-ulcer activity is due to steroids[10]. Petroleum ether extract of leaves of *A. pindrow* contains both glycoside and steroid constituent which give anti-inflammatory and anti-ulcer activity respectively[11]. In light of this, the present study was undertaken to evaluate the effect of *A. pindrow* leaves extract in dextran sulphate sodium induced ulcerative colitis (UC) in rats.

Materials and Methods

Plant collection and authentication

A. pindrow leaves were purchased from the wholesale supplier of traditional *Unani* medicine, Ballimaran, Delhi, India. Authentication of the plant sample was done by Dr. V. S. Thaker, Professor, Department of Bioscience, Saurashtra University, India.

Plant extraction

Leaves of *A. pindrow* were washed thoroughly with water and shade dried at room temperature. After drying chopped into pieces and then powdered material was extracted with petroleum ether by a soxhlet apparatus.

Preliminary phytochemical screening[16]

Preliminary phytochemical screening was performed in the petroleum ether extract of *A. pindrow* for the presence of alkaloids, glycosides, tannins, flavonoids, saponins, steroids, carbohydrates and amino acid.

LC-MS/MS analysis of petroleum ether extract of leaves of *A. pindrow*

An efficient liquid chromatography–electrospray ionization–tandem mass spectrometry (LC–ESI–MS/MS) method was used for identification and simultaneous determination of pinitol in leaves of *A. pindrow.*

Animals

An experiment was conducted according to the CPCSEA guideline and the study was approved by the institutional animal Ethics Committee. (Protocol no. BKMGPC/IAEC19/RP27/2017). Rats (200-250 g) were used and maintained under standardized condition. They were provided with a pelleted diet and purified drinking water. Throughout the experiments, animals were processed according to the suggested ethical guideline for the care of laboratory animals. Animals were divided into groups as shown in Table 1.

Dextran sulphate sodium (DSS) induced ulcerative colitis[17]

The administration of dextran sulphate sodium causes erosion with complete loss of surface epithelium because of its direct toxic effect on epithelial cells. It causes deformation in epithelial integrity, thereby increases the colonic mucosal permeability allowing penetration of large molecules such as DSS with molecular weight up to 50KD. DSS causes disturbance in the metabolism of phospholipids mainly phosphor-choline and glycerophosphocholine in the colon. These two phosphocholine and glycerophosphocholine are the most important metabolites of cholines and the major cellular constituents required for the assembly of biological membranes and disturbance in the metabolism suggest the possibility of destroyed membrane integrity in the presence of DSS. DSS significantly causes an increase in the production of all the proinflammatory cytokines in both the mid and the distal colon. But DSS induced ulcerative colitis appears to be more severe in the distal colon.

Change in body weight[17]

Body weights of all animals were measured from day 1 to day 11. Reduction in body weight is a sign of the generation of disease.

Disease activity index[17]

DAI was derived from the three major clinical signs (weight loss, diarrhoea and rectal bleeding). DAI is defined as: DAI = weight loss + diarrhoea score + rectal bleeding score.

Weight loss: body weight loss was calculated as the difference between the predicted body weight and actual body weight on a particular day.

Diarrhoea: the appearance of diarrhoea was defined as mucus/faecal material adherent to anal fur. The presence or absence of diarrhoea was scored as either 1 or 0 respectively.

Rectal bleeding: the appearance of rectal bleeding was defined as diarrhoea containing blood or mucus or frank rectal bleeding. The presence or absence of rectal bleeding was scored as either 1 or 0 respectively.

Change in colon length[18]

On day 11, animals were sacrificed and colons were isolated and their lengths were measured. Animals having colitis showed a tendency to shorten colon length.

Colon mucosal damage index[18]

On day 11, animals were sacrificed and colons were isolated. Colons were exposed and microscopic scoring was done as follows:0 = normal mucosa, no damage; 1 = mild hyperemia and oedema no erosion and ulcer; 2 = moderate hyperemia and oedema, erosion occurs on the mucosal surface; 3 = sever hyperemia and oedema, necrosis and ulcer appearing on the mucosal surface with major ulcerative area < 1 cm, and; 4 = severe hyperemia and oedema, necrosis and ulcer appearing on the mucosal surface with major ulcerative area > 1 cm.

Myeloperoxide (MOP) activity[19]

MPO activity helps in the assessment of polymorphonuclear cell (PMN) infiltration as a

Table 1 — Grouping of animals

Group no.	Group		Treatment
1	Vehicle control group		Normal saline (Orally) daily for 11 days
2	Diseased control group		2% W/V dextran sulphate sodium (DSS) in drinking water at day 5 to day 11
3	Standard treatment group		Sulphasalazine (dose- 50 mg/kg, orally) daily for 11 days + 2% DSS in drinking water at day 5 to day 11
4	Test group	Petroleum Ether Extract of leaves of *A. pindrow* (PEEAP)	PEEAP (100 mg/kg) orally daily for 11 days + 2% DSS in drinking water at day 5 to day 11
5			PEEAP (200 mg/kg) orally daily for 11 days + 2% DSS in drinking water at day 5 to day 11
6			PEEAP (400 mg/kg) orally daily for 11 days + 2% DSS in drinking water at day 5 to day 11

No of animals in a group=6

measure of inflammatory injury of the colon. Higher MPO activity is found in disease control groups. About 100 mg of colon mucosal scrapings were homogenized in one solution containing 0.5% DTAB dissolved in 50 mM potassium phosphate buffer (pH 6) and sonicated in an ice bath for 10s. The homogenates were freeze-thawed thrice and centrifuged for 15 minutes at 20,000×g. The level of myeloperoxidase (MOP) activity was measured spectrophotometrically. About 0.1 mL of the supernatant was mixed with 2.9 mL of 50 mM phosphate buffer, containing O-dianisidine dihydrochloride (0.167%) and 0.0005% hydrogen peroxide. The change in absorbance at 460 nm was measured for 1 minute at 15 seconds interval using a spectrophotometer.MOP activity was measured using the following formula:

MPO activity U/mg = X/Weight of tissue taken (mg)

where

$$X = 10 \times \frac{\text{change in observation per minute}}{\text{Volume of supernatant}}$$

Antioxidant parameters

***Malondialdehyde measurement*[20]**

The level of malondialdehyde (MDA) in the colon was determined as an indicator of lipid peroxidation. About 1 mL of supernatant was mixed with 2 mL of 10% (W/V) tri-chloroacetic acid and stood in ice for about 15 minutes. The precipitates were separated by centrifugation and 2 mL sample of the clear supernatant solution were mixed with 2 mL of aq. 0.67% thiobarbituric acid and heated in a boiling water bath for 10 minutes. The solution was then cooled in ice for 5 minutes after the absorbance was measured at 535 nm against an appropriate blank solution. The amount of malondialdehyde (MDA) (thiobarbituric acid reactive material) was calculated using the molar extinction coefficient 1.49×10^5 $m^{-1}Cm^{-1}$

***Super oxide dismutase (SOD)*[21]**

Although superoxide anion is a week oxidant, it ultimately produces powerful and dangerous hydroxyl radicals as well as singlet oxygen, both of which contributes to oxidative stress. The supernatant (0.1mL) of the sample was mixed with 0.1mL EDTA (1×10^{-4} M), 0.5mL of carbonate buffer (pH 9.7) and 1mL of Epinephrine (3×10^{-3} M). The optical density of formed adrenochrome was read at 480 nm for 3 minutes at an interval of 30seconds. the enzyme activity is defined as the concentration required for the inhibition of the adrenochrome production by 50% in one minute under the defined assay conditions.

***Catalase*[22]**

The homogenate was diluted 20 times with phosphate buffer. Read the absorbance of the sample containing 2mL distilled homogenate and 1mL H_2O_2 at 25 ^oC against a blank containing phosphate buffer instead of homogenate. The reaction was started by the addition of H_2O_2. Reading was taken for 3 minutes with 30 seconds interval. The difference in absorbance per unit time was expressed as the activity. One unit was defined as the amount of enzyme required to decompose 1.0 M of hydrogen peroxide per minute at pH 7.0 and 25 ^oC.

***Total protein*[23]**

The assay is based on polypeptide chelation of cupric ion (coloured chelate) in strong alkali tubes. Added 1 mL of homogenates to 4 mL biuret reagent. Incubated for 20 minutes at room temperature. Read the absorbance at 550nm against the reagent blank. The amount of total protein was calculated using the standard graph of bovine albumin. Bovine serum albumin ($A_{550}= 2.26*10^{-4}(ug/mL)^{-1}cm^{-1}$).

Histopathology[24]

The tissue sample from the colon was fixed overnight in 10% natural buffer formalin and was processed, sectioned (4 µm thick) and stained with hematoxylin and eosin. Each sample was observed and evaluated.

Statistical Analysis

Data are expressed as means ± standard deviations (SD). Statistical comparisons were made with one-way analysis of variance (ANOVA). P <0.05 was considered statistically significant.

Results

% Yield of Petroleum Ether Extract of Leaves *A. pindrow*

The % Yield of petroleum ether extract of leaves *A. pindrow* was 2% W/W.

Preliminary phytochemical investigation

The petroleum ether extract of leaves of *A. pindrow* was subjected to phytochemical investigation. The results revealed the presence of carbohydrate, steroids, glycosides, flavonoids, saponin, protein, and amino acid (Table 2).

LC-MS/MS analysis of the extract

Mass data for bioactive compounds present in petroleum ether extract of *A. pindrow* extract is

Table 2 — Qualitative phytochemical evaluation of PEEAP

S. No.	Phytochemicals	Chemical test	Result
1	Flavonoids	Shinoda test	-
		Alkaline reagent test	-
2	Carbohydrates	Molisch's test	-
		Benedict's test	-
		Barfoed's test	-
3	Proteins and Amino acids	Millon's test	-
		Ninhydrin test	-
		Biuret test	
4	Glycoside	Legal test	+
5	Tannins	Ferric chloride test	+
6	Alkaloids	Mayer's test	-
		Dragendroff's test	-
		Wagner's test	-
		Hager's test	-
7	Saponins	Foam test	-
8	Steroids	Libermann- Burchard test	+
		Salkowski's test	+

shown in Fig. 1 and 2. The chromatogram showed separation of different component present in petroleum ether extract of *A. pindrow*.MS chromatogram of above mention graph showed the MS spectrum of petroleum ether extract of *A. pindrow*. At 195.2 m/z, the peak of pinitol was observed (Fig. 3). The retention time of MS spectra of pinitol was 10.716 minutes using Gemini C18 (250mm x 4.6mm, 5µM) column and Water: Methanol (50:50) as eluent.

Effect of the extract on DSS induced ulcerative colitis in rats

Change in body weight

A significant drop in body weight was observed in the diseased group as compared to the normal group. PEEAP (400 mg/kg) and standard (50 mg/kg) treated group showed a lesser reduction in body weight as compared to the disease control group.

Change in colon length

There is a significantly lower colon length in the disease group (12.58±0.71 cm, P <0.05) as compared to the normal group (18.81±0.65 cm, P <0.05) as seen in Fig. 4 and Table 3. Animals treated with PEEAP (400 mg/kg) showed significantly higher colon length (4.93±0.51 cm, P <0.05) as compared to those of disease group animals (12.58±0.71 cm, P <0.05).

Colon mucosal damage index

The colon mucosal damage index score was significantly higher in the disease control group (3.66±0.21, P <0.05) as compared to those of the normal control group (P <0.05) as seen in Table 3. Animals treated with PEEAP (400 mg/kg) showed a

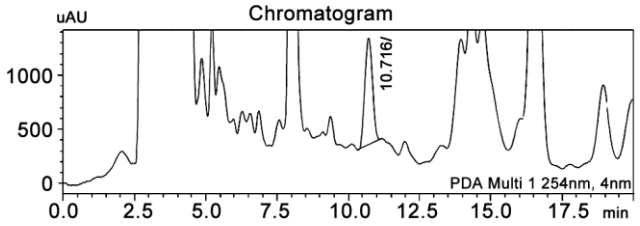

Fig. 1 — Chromatogram of petroleum ether extract of *A. pindrow*

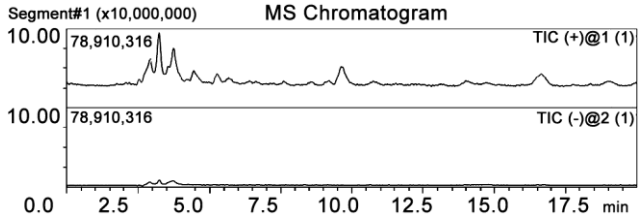

Fig. 2 — MS chromatogram of petroleum ether extract of *A. pindrow*

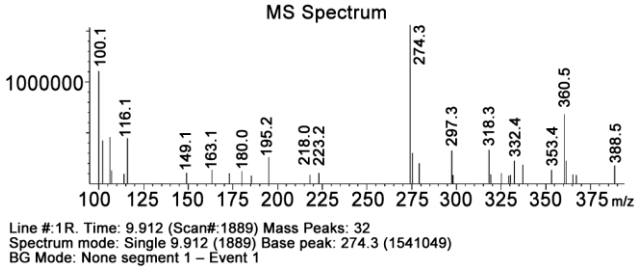

Fig. 3 — Mass peak of constituents present in petroleum ether extract of *A. pindrow*

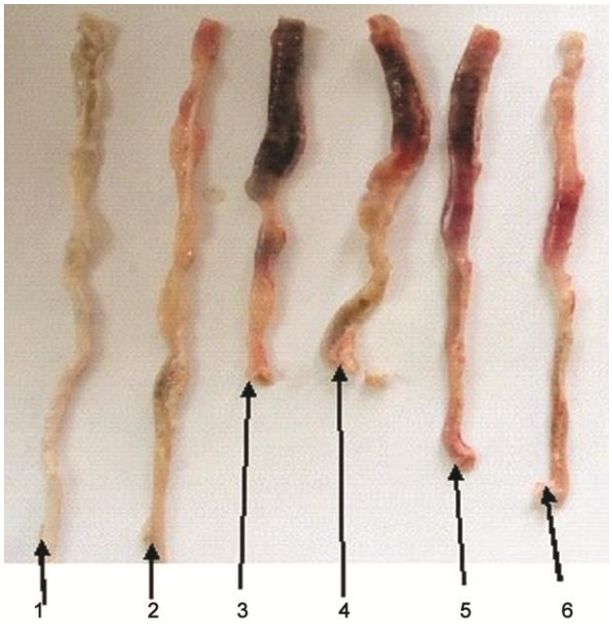

Fig. 4 — Effect of petroleum ether extract of leaves of *A. pindrow*on colon length. 1) Normal control group, 2) Std. (Sulphasalazine) treatment, 3) Disease group, 4) PEEAP (100 mg/kg) treatment, 5) PEEAP (200 mg/kg) treatment, and 6) PEEAP (400 mg/kg) treatment

Table 3 — Effect of petroleum ether extract of *A. pindrow* on colon length, CMDI score, MPO level, MDA level, SOD level and catalase level

Groups	Colon length (cm)	CMDI score	MPO (U/ mg of protein)	MDA (μM/g of protein)	SOD (U/mg of protein)	CAT (U/mg of protein)
Normal control	18.81±0.65	-	0.066±0.014	0.57±0.12	63.61±9.64	2.33±0.44
Disease control	12.58±0.71#	3.66±0.21#	0.100±0.008#	1.00±0.08#	21.04±1.19#	0.35±0.09#
Std. treatment (sulphasalazine)	15.53±0.73*	1.16±0.16*	0.067±0.007*	0.65±0.06*	43.75±5.20*	1.97±0.26*
PEEAP treatment (100 mg/kg)	12.11±0.82	3.00±0.25*	0.096±0.007	0.91±0.07	22.38±2.89	0.56±0.10
PEEAP treatment (200 mg/kg)	14.13±0.35	2.16±0.14*	0.087±0.006	0.87±0.06	28.28±2.73*	0.77±0.10
PEEAP treatment (400 mg/kg)	14.93±0.51*	1.33±0.21*	0.079±0.010*	0.79±0.16*	42.94±6.46*	0.86±0.22*

All the values are expressed in mean ± SEM (n= 6); # Significantly different from normal (P <0.05); *Significantly different from control (P <0.05)

significantly lower CMDI score (1.33±0.21, P <0.05) as compared to those of the disease control group (3.66±0.21, P <0.05).

Disease Activity Index

Animals treated with DSS showed a significant increase in DAI score from day 7 to 11 as compared to the normal group (Fig. 5). Animals pre-treated with PEEAP (400 mg/kg) showed significantly lower DAI score (2.33±0.61, P <0.05) as compared to those of disease group animals (9.0±0.37, P <0.05) on the 11th day.

Myeloperoxidase (MPO) activity

As an index of neutrophil migration, MPO activity was measured and it was found to be significantly higher in the disease control group (0.10±0.01, P <0.05) as compared to the normal group (0.07±0.01, P <0.05)as seen in Table 3. In the case of PEEAP (400 mg/kg) treated group, the MPO activity was significantly reduced (0.07±0.01, P <0.05) as compared to the disease control group (0.10±0.01, P <0.05).

Malondialdehyde (MDA) level

Tissue MDA level was measured and it was found to be significantly higher in the disease control group (1.00±0.08, P <0.05) as compared to the normal group (0.57±0.12, P <0.05) as seen in Table 3. In the case of PEEAP (400 mg/kg) treated group, the MDA activity was significantly reduced (0.79±0.16, P <0.05) as compared to the disease control group (1.00±0.08, P <0.05).

Super oxide dismutase (SOD)

Effect of petroleum ether extract of leaves *A. pindrow* on SOD level was measured and it was found to be significantly lower in the disease control group (21.04±1.19, P <0.05) as compared to the normal group (63.61±9.64, P <0.05)as seen in Table 3. In the case of PEEAP (400 mg/kg) treated group, the SOD activity was significantly increased (42.94±6.46, P <0.05) as compared to the disease control group (21.04±1.19, P <0.05).

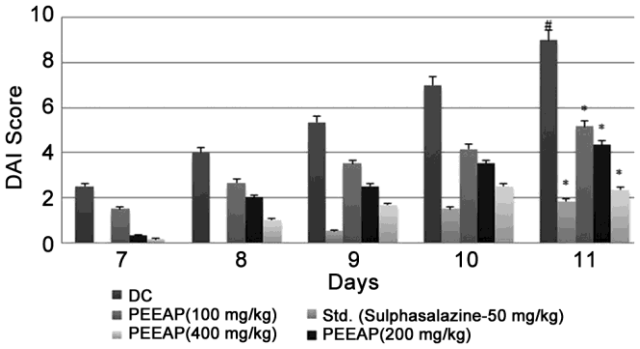

Fig. 5 —Effect of petroleum ether extract of *A. pindrow* on disease activity index score.
All the values are expressed in mean±SEM (n= 6); # Significantly different from normal (P <0.05); *Significantly different from control (P <0.05).

Catalase activity

Effect of petroleum ether extract of leaves of *A. pindrow* on catalase activity was measured and it was found to be significantly lower (0.35±0.09, P <0.05) in the disease control group as compared to the normal control group (2.33±0.44, P<0.05)as seen in Table 3. In the case of PEEAP (400 mg/kg) treated group, the catalase activity was significantly increased (0.86±0.22, P<0.05) as compared to the disease control group (0.35±0.09, P <0.05).

Histopathology

Histological finding of the disease group (DSS treated) showed transmural inflammation with

extensive necrosis, ulceration, and increased infiltration of polymorphonuclear leukocytes, lymphocyte and. presence of cryptic abscesses which resembles the histological feature of human UC.

PEEAP (400 mg/kg) treated group and standard (50 mg/kg) treated group showed reversal of mucosal damage as compared to the disease group as evidence by less cell infiltration as compared to disease group as shown in Fig. 6.

Discussion

A. pindrow is a well-known plant drug in *Ayurvedic* and *Unani* medicines. In *Ayurveda*, it is used as a remedy for fever, respiratory, and inflammatory ailments. Anti-diabetic, anti-inflammatory, analgesic, hypnotic, and anti-ulcer activities in rats have been reported for extracts and various fractions from *A. pindrow* leaves. It is one of the primary herbs of the ayurvedic system and finds its position as a versatile plant having a wide spectrum of medicinal properties[11].

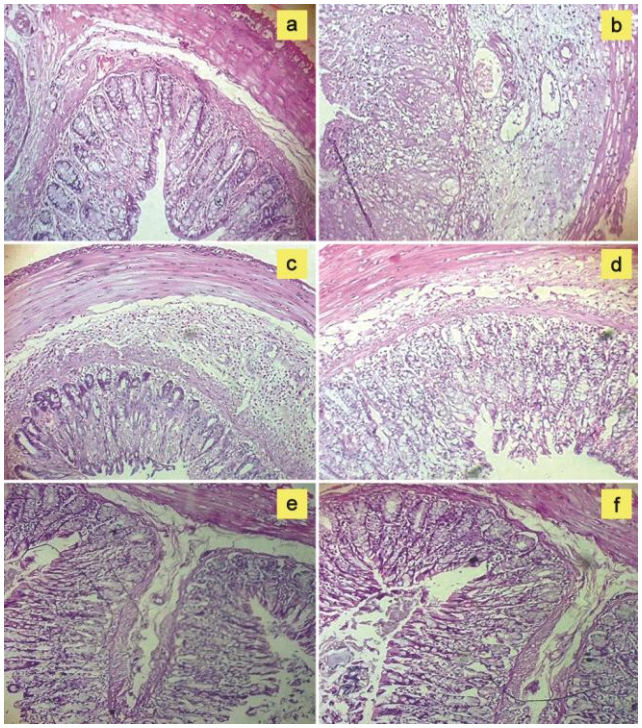

Fig. 6 —Histopathology study of the colon. a) Normal group, normal mucosal structure, b) Disease group, transmural inflammation with extensive necrosis, ulceration and cell infiltration, c) Standard treatment group (sulphasalazine – 50 mg/kg) normal colonic structure with mucosal infiltration only, d) PEEAP (100 mg/kg group) transmural inflammation and epithelial damage cell infiltration, e) PEEAP (200 mg/kg) group) Near normalization of structure with mucosal infiltration only, and f) PEEAP (400 mg/kg) normal mucosal structure with lesser cell infiltration.

Previously, *A. pindrow* and its constituents have been reported to having anti-inflammatory[12], anti-ulcer[25], and anti-oxidant activity[26,27]. One of the active constituents of this plant, pinitol is reported to suppress NF-κB[11].

Ulcerative colitis is a chronic inflammatory disorder of the gastrointestinal tract affecting mainly the colon part. DSS induced ulcerative colitis model was used for the present investigation. Randhava *et al.* reported that 2-5% DSS ingestion by oral route for 7 days altered distal ileum morphological structure, with an increased ileum crypt depth and crypt cell proliferation which resembled the UC in human[17].

Reduction in body weight is a sign of the generation of colitis. Animals treated with DSS showed a tendency to shorten colon as compared to those of a normal control group[17]. Reduction in body weight was observed in the disease group as compared to the normal group. PEEAP (400mg/kg) showed a lesser reduction in body weight as compared to the disease control group.

A significant increase in DAI score for disease control group animals on the final day was observed[18]. Animals treated with DSS shows significant weight loss, rectal bleeding, and diarrhoea as compared to those of the normal group. Animals treated with PEEAP (400 mg/kg) showed a significant reduction in DAI score to those of the disease control group.

A further assessment of disease by CMDI scoring in the disease control group showed bowel wall thickening, adhesion to surrounded tissue as well as erosion, oedema, and small patches of ulcer in colon[17]. The result of the CMDI score suggested that PEEAP (400 mg/kg) showed significant protection from macroscopic damage of the colon in this study.

Histological finding of the disease group (DSS) showed transmural inflammation with extensive necrosis, ulceration and increased infiltration of polymorphonuclear leukocytes, lymphocytes and the existence of cryptic abscesses which resemble histological feature of human UC[28]. PEEAP treated group (400 mg/kg) showed normal mucosal structure with cell infiltration as compared to the disease control group.

Myeloperoxidase (MOP) is an enzyme mainly found in azurophilic granules of neutrophils. It can serve as a good marker of inflammation, tissue injury and neutrophil infiltration in gastrointestinal tissues[19].

Pre-treatment with PEEAP (400 mg/kg) showed a decrease in polymorphonuclear infiltration demonstrated by a significant reduction in MOP activity.

Malonaldialdehyde (MDA) is considered an important indicator of lipid peroxidation, which is found to be higher in the disease control group as compared to the normal control group[20]. This might be due to lipid peroxidation. Rats treated with PEEAP (400 mg/kg) showed protection against lipid peroxidation characterized by a significantly reduced level of MDA.

Oxidative damage may represent a crucial pathogenic factor in UC because intestinal inflammation is accompanied by increased production of reactive oxygen and nitrogen species. Oxidative stress is believed to play a key role in the pathogenesis of UC-related intestinal damage. Intestinal mucosal damage in the UC is related to both increased free radical production and a low concentration of endogenous antioxidant defence[7].

The antioxidant enzyme, mainly superoxide dismutase and catalase are the first line defence enzyme against free radicals[24]. In the present study, it was observed that the PEEAP (400 mg/kg) significantly increased antioxidant parameter (CAT and SOD) as compared to the DSS group. This shows that the PEEAP can reduce reactive free radicals that might lessen oxidative damage to the tissue.

In the present study, there was a lower level of catalase (CAT) and superoxide dismutase (SOD) with the concomitant higher level of malondialdehyde (MDA) and myeloperoxidase (MPO) concentration in the homogenized colonic tissue sample after DSS administration.

The present study shows that treatment with PEEAP (400 mg/kg) is found to be protective against the progression of ulcerative colitis.

Conclusion

The present investigation reveals that petroleum ether extract of leaves of *A. pindrow* is potentially beneficial as a curative agent in experimental ulcerative colitis induced by DSS. Phytochemical screening showed the presence of glycoside and steroid which may be responsible for the anti-inflammatory and anti-ulcerogenic activity respectively. While flavonoids and phenols may be responsible for the antioxidant activity of the plant. The animal treated with PEEAP (400 mg/kg) showed significant higher colon length than the disease control group. CMDI was found to be significantly lower in the treatment group as compared to the disease group. While DAI was found significantly lower in the treatment group as compared to the diseased control group. It also showed a lower level of MPO and MDA and a higher level of SOD and catalase than those observed in the disease group. Histopathologically, it showed normal mucosal structure with cell infiltration. Further studies are warranted to isolate the active principle responsible for the activity and determination of the molecular mechanism involved for its beneficial role in IBD.

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Conflict of interest

The authors declare that there is no conflict of interest.

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