Original Article

Hemidesmus indicus var. *pubescens* Root Ameliorates Dextran Sodium Sulfate-induced Ethanol Augmented Ulcerative Colitis in Rats

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ABSTRACT

Aim: Ulcerative colitis is a chronic, idiopathic, inflammatory condition of the colon. Studies have indicated that *Hemidesmus indicus* var. *pubescens* root extract possess antioxidant and antiulcerogenic activity. In this study, we aimed to evaluate the therapeutic effect of *Hemidesmus indicus* var. *pubescens* aqueous root extract in Dextran Sodium Sulfate induced, ethanol augmented ulcerative colitis in Wistar rats. **Materials and Methods:** The animals were administered with 2% Dextran Sodium Sulfate for 3 consecutive days orally followed by intracolonic instillation of 30% ethanol on 4th day. These animals were randomly divided into 4 groups viz.: Disease, Low dose extract -200 mg/kg, High dose extract -400 mg/kg and Sulfasalazine-500 mg/kg while healthy, untreated rats were assigned as the normal control group. Treatment with the extract and the standard was initiated on day 5, continued throughout the study period of 21 days. The effect of extract on Ulcerative Colitis was determined by evaluating haematological parameters, disease activity index, ulcer index, colon and spleen weight, biochemical parameters (malonaldehyde, glutathione, Catalase) and histopathological studies. **Results:** Animals treated orally with extract showed a reduction in Disease activity Index and Ulcer Index compared to disease control. Moreover, a significant decrease ($p<0.01$) in malonaldehyde and a significant increase ($p<0.01$) in catalase and glutathione levels compared to the disease group were also observed. Histopathological examination also showed augmentation of colonic architecture on treatment with extract, all of which confirmed the protective effect of extract against Dextran Sodium Sulfate- ethanol-induced Ulcerative Colitis. **Conclusion:** These results suggest that *Hemidesmus indicus* var. *pubescens* root was able to attenuate Dextran Sodium Sulfate induced ethanol augmented ulcerative colitis indicating its anti-ulcerative colitis effects.

Key words: Dextran Sodium Sulphate – Ethanol, *Hemidesmus indicus* var. *pubescens*, Indian sarasaparilla, Ulcerative colitis, Inflammatory Bowel Disease.

INTRODUCTION

Ulcerative colitis (UC) and Crohn's disease (CD) is the long-lasting inflammatory condition which belongs to inflammatory bowel disease. IBD was considered as western disease however studies show its increased occurrence in Asian countries. Epidemiological data indicate that the incidence of UC in Asian countries is twice as that of CD. Two population-based studies reported a higher prevalence (42.8/10000 and 44.3/10000) of UC in India.[1] The precise cause of UC is unknown; nevertheless, it is hypothesized that oxidative stress and immunological dysfunction plays a crucial role in the development of UC. Disruption of the antioxidant system of intestinal mucosa leads to oxidative damages which cause mucosal ulceration and destruction of mucosal barriers. This accelerates the development of the disease by exposing the mucosal immune system to the luminal antigens.[2] Besides, environmental, microbiological and multiple hereditary factors can also contribute to the pathogenesis of UC.[3-6]

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In UC, the selection of therapy is based on the severity of the disease, disease location, side effects and the cost of the drug. Mostly anti-inflammatory drugs, immunomodulators, steroids and antibiotics can be used as palliative therapy for the management of UC.[7] However, most of these drugs have synthetic origins and are observed to produce several side effects; hence the use of these drugs is debatable owing to its uncertain safety and efficacy issues. As of now, there is no efficient therapy for the complete cure of UC apart from proctocolectomy. This demands the development of newer and safer molecules with substantially good effects in treating UC.

Indian Sarsaparilla is a key drug used in Indian traditional medicine, bestowed with numerous medicinal properties. The accepted botanical source of Indian Sarsaparilla is *Hemidesmus indicus* var. *indicus* belonging to the family Periplocaceae. In the traditional system of medicine, the root of *Hemidesmus indicus* var. *indicus* is useful in dysentery, diarrhoea, epilepsy, skin diseases, syphilis, bronchitis, fever, asthma, eye diseases, leprosy and leukoderma.[8] In addition, ayurvedic literature also describes its use as anti-spasmodic, anti-atherogenic, anti-inflammatory, immune and memory enhancing agents. *Hemidesmus indicus* (L.) R.Br. var. *pubescens* (Wight and Arn) Hk. f. is a taxonomic variety commonly found in South India.[9]

Though this variety is used as a substitute for *indicus* in various ayurvedic formulations, it is under-studied for its potential therapeutic activity. It consists of tannins, glycoside, phenolic compounds and coumarins as its major phytoconstituents. The presence of smilagenin, sarsapogenin and β-sitosterol, have also been reported in *Hemidesmus indicus* var pubescens aqueous root extract (HIAR). Various pharmacological studies such as the antimicrobial, antiepileptic, antipyretic, antiulcerogenic and hepatoprotective effect of HIAR have also been reported.[10-14] Madhuri *et al*., reported anti-ulcerative colitis activity of ethanolic extract of *Hemidesmus indicus* combined with *Ocimum basilicum* in DSS induced colitis in mice. Since no study has been reported on the anti-ulcerative colitis potential of *Hemidesmus indicus* var pubescens aqueous root extract, the present study was undertaken.[15] Additionally, *Hemidesmus indicus* var. *pubescens* is known to exert antioxidant and free radical scavenging effects that make it a potential candidate for this study.

MATERIALS AND METHODS

Collection of Plant material

The root of *Hemidesmus indicus* var. *pubescens* was collected from Thiruvananthapuram District, Kerala, India. The plant material was identified, authenticated and herbarium specimen (Specimen No.034) has been prepared and deposited in the herbarium, Department of Pharmacognosy, Faculty of Pharmacy, MSRUAS, Bengaluru.

Preparation of Aqueous extract

The roots of *Hemidesmus indicus* (L.) R. Br. var. *pubescens* (Wight. And Arn.) Hook. f. was cleaned, dried under shade and coarsely powdered. About 50 g of powdered material was subjected to the extraction process by maceration with 500 mL of distilled water for 48hr at room temperature. The extract was filtered with Whatman No. 1 filter paper and concentrated to dryness. This was reconstituted in distilled water to the required doses of 200 and 400 mg/kg body weight before administration to experimental animals.[16,17] Doses of the extract were chosen for the study based on the acute toxicity studies earlier reported in our laboratory.[14]

Animals

Wistar rats of either sex weighing 150–200 g obtained from Veterinary College, Hebbal and Bengaluru, India was used for this investigation. The animals were maintained under the standard condition as per the guidelines of Committee for the Purpose of Control on Experiments in Animals (CPCSEA) in the faculty animal house facility, Faculty of Pharmacy, MSRUAS, Bengaluru and Karnataka. The animals used for this study protocols have been approved by the Institutional Animal ethical committee (IAEC) Approval no. Reg. No: XXI/MSRFPH/GP-15/12.09.2018.

Induction of colitis

UC was induced in animals by administering DSS (2% w/v) orally in drinking water for 3 consecutive days. On 4th day overnight fasted animals were instilled with 0.5 ml of 30% ethanol intracolonically by using a flexible plastic catheter (OD, 2 mm) under diethyl ether-induced light anesthesia. Animals were maintained in a head-down position for a few seconds to prevent leakage of solution from the colon. The animals showing evident weight loss and altered stool consistency were considered positive for UC and were included in the study.[18]

Experimental design

After the confirmation of UC, total of 30 *Wistar* rats were randomly divided into five groups of six rats each. The treatment groups were administrated with standard (Sulfasalazine, 500 mg/kg/day), HIAR (200 mg/kg and 400 mg/kg) for 21 days after colitis induction. Animals were allotted into the following groups - Control group:

0.5% saline orally once daily for 21 consecutive days (without colitis induction)
Disease control group: 2% w/v DSS + 0.5 ml of 30% ethanol
Low dose: 2% w/v DSS + 0.5ml 30% ethanol + HIAR 200 mg/kg/day
High dose: % w/v DSS + 0.5ml 30% ethanol + HIAR 400 mg/kg/day
Standard: 2% w/v DSS + 0.5ml 30% ethanol + Sulfasalazine 500mg/kg/day
After 24 hr of final treatment with HIAR and standard drug, overnight fasted animals were anesthetized with light ether and retroorbital blood samples (1.5 mL) were withdrawn using heparinised microcapillaries to evaluate the haematological parameters. Animals were then euthanized by cervical dislocation. Distal colon and spleen were isolated from each animal and weighed. Colon was further used to determine ulcer index, biochemical and histopathological studies.

Effect of HIAR in the severity of colitis

Determination of Disease Activity Index (DAI)

DAI was determined by the Shi-Ying Wang method with slight modification. In this method, the DAI scale was used to assess the severity of colitis.[19] Each animal was closely monitored and scored for weight changes, symptoms like loose stools and rectal bleeding throughout the study period (before, during and after treatment) and the sum of these 3 sub scores constituted the overall severity of the disease. (Table 1)

Determination of Ulcer Index (UI)

The severity of inflammation and ulcer was observed macroscopically according to the following scale: 0 - no macroscopic change; 0.5 - mucosal erythema; 1- mild mucosal edema and erythema; 1.5- moderate edema, slight ulcers or erosions; 2 - severe ulceration, erosions, edema and tissue necrosis.[20]

Determination of colonic oxidative stress markers

Malondialdehyde (MDA)

1ml of TBA:TCA:HCl was added to 500 µL of 10% w/v of colon homogenate prepared using potassium chloride (0.15 M). Further the mixture was boiled for 15 min, then cooled and centrifuged at 10,000 rpm for 5 min. The supernatant solution was separated and absorbance were measured at 532 nm against reagent blank. The MDA content was calculated as thiobarbituric acid reacting substance (TBARS) and expressed in terms of nmol/100 mg of tissue, using molar extinction co-efficient, 1.56×10^5 moles/cm.[21,22]

Table 1: Scoring of the disease activity index.

Score	Weight loss	Stool consistency	Rectal bleeding
0	None	None	No bleeding
1	0-10%	Wet	Traces of blood in the stool
2	10-20%	Soft	Blood around anus
3	> 20%	Watery	Gross Bleeding

Catalase

1.9 ml of phosphate buffer (pH 7) was added to 100 µL of 10% w/v colon homogenate prepared by 0.15 M potassium chloride buffer. Followed by the addition of 1 ml of 10 mM H_2O_2 solution, the absorbance was taken at 240 nm at 0 min. The decrease in the absorbance after 1 min of addition of 1 ml of H_2O_2 was noted again. Catalase activity was calculated by using molar extinction coefficient of H_2O_2 and expressed in terms of U/mg of wet tissue. [23,24]

Glutathione (GSH)

10% w/v of colon homogenate prepared with sucrose in phosphate buffer (pH 7.4) was used for the estimation of glutathione (GSH) by the method of Paglia and Velentine, 1967. 200 µl of Ellman's reagent was added to this reaction mixture and after 5 min, absorbance was read at 412 nm. The amount of GSH was determined and expressed in terms of n mol/ 100 mg of tissue.[25]

Statistical analysis

The results of parametric data were expressed as Mean ± SD and statistically analyzed by one-way ANOVA followed by Dunnett's test. All non-parametric data were analyzed by the Kruskal-Wallis test followed by Dunnett's test.

RESULTS

Hematological parameters

The hematological parameters of different groups of animals in the study are depicted in Table 2, which shows that the levels of WBC and neutrophils in disease group were significantly (p<0.01) elevated while the levels of RBC, lymphocytes, hemoglobin and platelets were decreased significantly (p<0.01) when compared to the normal control animals. This alteration in the hematological values indicates inflammatory changes induced by DSS and ethanol. On the other hand, treatment with HIAR and the standard drug showed remarkable changes (p<0.01) in hematological values.

Table 2: Effect of HIAR on haematological parameters of DSS-Ethanol induced ulcerative colitis in *Wistar* rats.

Parameters	RBC count ($x10^6$/µL)	WBC Count ($x10^3$/µL)	Neutrophils %	Lymphocytes %	Haemoglobin g/dl	Platelet Count ($x10^3$/µL)
Normal Control	7.134 ± 0.28	9.5 ± 0.54	16.98 ± 0.01	84.33 ± 0.14	12.64 ± 0.36	601.16 ± 21.04
Disease Control (DSS)	5.140 ± 0.15#	13.6 ± 0.21 #	30.38 ± 0.10#	68.64 ± 1.87#	8.14 ± 1.36#	346.16 ± 53.67#
High Dose (400mg/kg)	7.312 ± 0.18*	9.6 ± 0.51*	17.61 ± 0.04*	78.84 ± 0.53*	14.91 ± 0.25*	611.22± 31.58*
Low dose (200mg/kg)	6.667 ± 0.19*	11.5 ± 0.54*	25.25 ± 1.53*	72.13 ± 0.53*	13.65 ± 1.21*	502.99 ± 38.41 *
Standard (Sulfasalazine, 500 mg/kg)	7.134 ± 0.28*	10.1 ± 0.63*	19.90 ± 0.55*	72.13 ± 0.68*	13.33 ± 1.36*	568.23 ± 38.51*

Note: Values are mean ± SD of six animals in each group. Data analysed by one way ANOVA followed by Dunnett's multiple comparison test.*p<0.01 when compared to Disease control. #p<0.01 compared to normal control

Effect of HIAR in the severity of colitis

Effect of HIAR in DAI and UI

DAI and UI grades represent the severity of the disease, which was used to assess the therapeutic effect of HIAR. For this, all the animals were closely monitored throughout the study period of 21 days. Animals in the normal group had no apparent change in stool consistency and body weight. However, animals treated with DSS-ethanol were listless and had a semi-dilute stool on day 5. By day 6, gross bloody stool and weight loss were observed in all groups, which indicates the induction of UC. Animals received HIAR treatment were effectively relieved off the symptoms and showed a reduction in DAI compared to disease control. Animals in the disease control group showed moderate edema, erythema and slight ulcers or erosions (Figure 1E). While the animals receiving HIAR and the standard drug showed no ulceration but had signs of mild mucosal edema and erythema (Table 3) (Figure 1B, C and D).

Figure 1: Morphological features of colons of different groups.

A-Normal, B-Standard, C-High Dose, D-Low dose, E-Disease
Note: Mild mucosal edema and erythema was found in B, C and D. Moderate edema, erythema and slight ulcers or erosions was found in E

Table 3: Effect of HIAR on DAI and UI of DSS-ethanol induced ulcerative colitis in *Wistar* rats.

Treatment	Disease Activity Index				Ulcer Index
	Day 1	Day 7	Day 14	Day 21	
Normal	0	0	0	0	0
Disease	0	6(0.5)#	7(0.5)#	8(0.5)#	1.5(0.5)#
High dose	0	4(1.5)	3(1.25*	2(1.25)*	1(1.25)
Low dose	0	5(0.5)	4(1.5)	3(0.5)	1(1.25)
Standard	0	3(1.25)**	2(1.25)**	1(0.5)**	0.5(0.5)**

Note: Scores are medians ± interquartile ranges of six animals in each group. Data analysed by the Kruskal-Wallis test followed by Dunnett's multiple comparison test. *p<0.05, **p<0.001when compared to disease control and #p<0.05 when compared to normal.

Colonic weight and spleen weight

Table 4 showed the mean ± SD values of colon and spleen weight. It was observed that the colon and spleen weight in the disease control animals showed a significant elevation compared to the normal control. Animals treated with HIAR 200 mg/kg and 400 mg/kg exhibited a significant reduction (p<0.01) in colon and spleen weight compared to the disease control group (Table 4).

Biochemical parameters

To determine the effect of HIAR on DSS-ethanol induced oxidative stress in the colon, MDA, GSH and Catalase level was measured. In the disease group, a significant elevation in MDA levels when compared to the normal group indicates the oxidative stress induced by DSS-ethanol. Treatment with HIAR (400 mg/kg) showed a notable reduction (p<0.01) in MDA compared to that of the disease control group. Glutathione and catalase levels in the disease control were found to be decreased significantly compared to the normal control group. On the other hand, HIAR (400 mg/kg and 200 mg/kg) showed a significant increase in the glutathione and catalase compared to the disease group (Table 5).

Table 4: Effect of HIAR on the spleen and colonic weight of DSS-Ethanol induced ulcerative colitis in *Wistar* rat.

Treatment	Colon weight (g)	Spleen Weight (g)
Normal	2.05 ± 0.07	1.05 ± 0.04
Disease	2.62 ± 0.16#	1.613 ± 0.03#
High Dose	2.03 ± 0.10*	1.38 ± 0.45*
Low Dose	2.40 ± 0.02*	1.30 ± 0.02*
Standard	2.06 ± 0.09*	1.16 ± 0.05*

Note: Values are mean ± SD of six animals in each group. Data analyzed by one way ANOVA followed by Dunnett's multiple comparison test.*p<0.01 when compared to DSS rats. #p<0.01 compared to normal.

Table 5: Effect of HIAR on colonic oxidative stress markers of DSS-Ethanol induced ulcerative colitis in *Wistar* rat.

Treatment	MDA (nM/100mg Tissue)	GSH (nM/100mg Tissue)	Catalase (U/mg Tissue)
Normal	3.087± 0.033	6.898 ± 0.079	6.623 ± 0.248
Disease	6.188 ± 0.017#	1.820 ± 0.066#	1.018 ± 0.203#
High dose	4.004± 0.061*	4.974 ± 0.030*	5.265 ± 0.270*
Low dose	6.011 ± 0.255	3.048 ± 0.061*	2.030 ± 0.235*
Standard	3.964 ± 0.069*	5.605 ± 0.037*	5.251 ± 0.121*

Note: The values are represented as a mean ± SD of six animals in each group. Data analysed by one way ANOVA followed by Dunnett's multiple comparison tests. *p<0.01 when compared to DSS rats. #p<0.01compared to normal

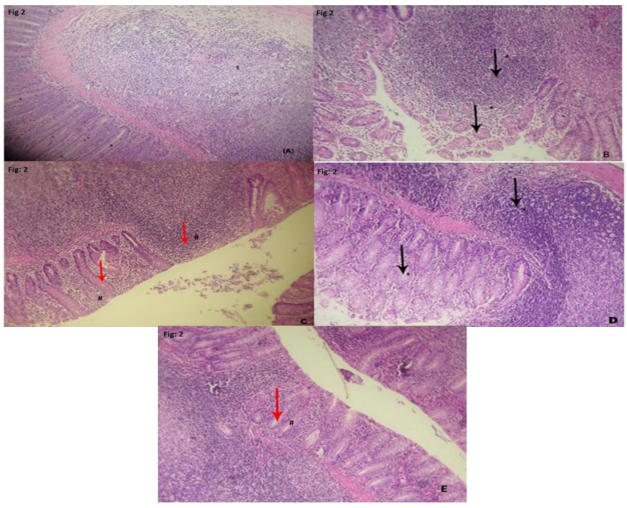

Figure 2: Histopathology.

A-Normal, B-Disease, C-High Dose, D-Low Dose, E-Standard

Note: Black arrows show severe inflammatory cell infiltration and crypt abscess. The red arrow shows mild inflammatory cell infiltration and crypt abscess.

Histopathology

Microscopically, the colon of animals treated with DSS-ethanol showed disruption of the glandular epithelial layer, infiltration of inflammatory cells and crypt abscess (Figure 2B) relative to the normal group (Figure 2A). However, animals treated with HIAR depicted a colon tissue with very mild glandular epithelial distortion indicates the potential of extract to treat cellular damage caused by the induction of disease. Moreover, mild crypt abscesses in high dose of HIAR treated animals, hypothesized that HIAR could reduce the mucosal damage by reinforcing its self-repair (Figure 2 C, D, E).

DISCUSSION

Oxidative stress is one of the important factors which is involved in the perpetuation of UC. It has been known for years that oxidative stress and associated inflammation in the colon paves a path for DNA damage, which may further increase the risk of colorectal cancer. It may also increase the probability of developing other extraintestinal cancers like enteropathy-associated T-cell lymphomas, non-Hodgkin's T-cell lymphomas, hepatobiliary and rectal carcinomas.[26] Because of its recurrent trend and protracted course WHO has identified UC as a modern refractory disease, which mainly affects the sigmoid colon, mucosa and submucosa of rectum based on its severity.[27] Patients with UC experience severe abdominal pain because of inflammation and ulceration of colon associated with anemia due to excessive rectal bleeding. Hence, the management of UC involves the use of immunomodulators, anti-inflammatory drugs and antibiotics which is associated with several side effects. The response of UC patients to conventional medications such as corticosteroids, thiopurines and immunomodulators is variable and can diminish over time.[28] Therefore, the need for a novel therapeutic agent is paramount. *Hemidesmus indicus* var. *pubescens* otherwise called Sveta Sariva used in traditional medicine possess great advantage due to its unique properties and biological safety. Ayurvedic literature depicts that the root of this plant has anti-atherogenic, memory enhancing, anti-spasmodic and anti-inflammatory properties. It is also reported to have immunostimulatory, neuroprotective, hepatoprotective, antidiabetic, antiulcerogenic, nephroprotective and cardioprotective activities.[29] Antiulcerative colitis activity of *Hemidesmus indicus* var. *pubescens* has not been reported so far, hence the present work was been undertaken.

Among various chemical models of UC, DSS induced UC experimental model is widely used due to its ability to induce colitis by causing epithelial damage. The morphological and symptomatical similarity of DSS induced colitis to human UC makes it more reliable than other models.[30] Moreover, DSS is found to be useful due to its simplicity in administration and ease of controlling the dosage.[31] Studies have shown that administration

of a lower dose of DSS (Colitis grade, MW = 36,000-50,000) followed by ethanol was able to induce severe acute colitis than DSS and ethanol alone. It was reported that ethanol acts as a "barrier breaker", that destroys the intestinal epithelium leading to the infiltration of pro-inflammatory cytokines. It causes the rapid development of ulceration associated with inflammation and the same can be augmented in the presence of DSS.[17] Additionally, oxidative stress induced by DSS on colon leads to the disruption of the antioxidant defense mechanisms, which may further lead to the production and accumulation of Reactive Oxygen Species (ROS). The inability of endogenous enzymatic and non-enzymatic antioxidants to handle the excess ROS leads to the inflammation, which in turn develops into extensive superficial mucosal ulceration. The imbalance between ROS and endogenous antioxidant mechanisms can be normalized by using natural antioxidants which is more beneficial than conventional drugs with a high toxic profile. In the present study, we identified ulceration associated with colorectal polyps and hyperemia in the colon specimen of disease group as an indication of the oxidative stress in the colon. Colon specimens of animals receiving HIAR treatment showed slight hyperemia and colorectal polyps without any ulceration. This marks the anti-inflammatory effect of HIAR that may be accounted for the presence of phytoconstituents like coumarins, glycosides and phenolic compounds, steroidal sapogenins (smilagenin and sarsapogenin) in the *Hemidesmus* root.

One of the important parameters to quantify the severity of UC is DAI, which is a clinical scoring system. DAI is the sum of the 3 subscores of body weight loss, change in stool consistency and gross rectal bleeding.[32] The main macroscopic characteristic of ulcerative colitis is symmetrical and contiguous inflammation in colonic mucosa, which is more diseased in distal colon compared to proximal colon and rectum. Based on the extent of disease UC is divided into left-side colitis, ulcerative proctitis, and pancolitis and sub-total colitis. Earlier studies have shown that 2% DSS for 3 days causes only loose stool without any macroscopic damages, but a single dose of ethanol intracolonically on 4th day in DSS treated animals induced macroscopic damage with diarrhea/loose stool, weight loss and bleeding. Similarly, in our study animals that received DSS-ethanol showed a significant reduction in weight on the 6th day and this weight loss is considered an important manifestation of human UC as well. The weight reduction could be due to malabsorption, maldigestion and diarrhea, a similar trend was also observed in acetic acid-induced UC in other studies.[33] Animals receiving HIAR also showed weight reduction that could be due to inherent weight reducing property of HIAR which is in agreement with previous reports.[34] During the study, mortality was seen in two animals of disease group on 5th day and 6th day due to excess rectal bleeding indicating the severity of colitis induced (the same number of animals were replaced to maintain the sample size). However, other groups did not show any signs of mortality.

Haematological investigation of the disease group showed a significant decrease in RBC and Hb, which could due to blood loss and poor absorption of iron in the intestine due to intestinal damage. Besides neutrophil and lymphocyte count provides an insight into the colitis state. A significant increase of those in the disease group indicates the activation of the inflammatory cell by DSS/ethanol treatment. Colon weight and macroscopic scoring of the ulcer was used as a reliable indicator for assessing the severity of UC.[35] A significant increase in colon weight and ulcer index in the DSS/ethanol group indicates edema and ulceration and on the other hand, a significant reduction in treatment groups showed the attenuation of inflammation by HIAR treatment. Splenic atrophy has been reported in association with UC, which was evident by the increased spleen weight in the disease group. Those animals which received HIAR treatment showed a significant decrease in spleen weight indicating the reduction of inflammation.

Besides, glutathione and catalase that prevent cellular damage from reactive oxygen species, MDA, a lipid peroxidation product is also widely used as a biomarker for oxidative stress.[36] Herein, a significant reduction of GSH and catalase with a subsequent increase in MDA levels in the disease group indicates the impairment of mucosal defense mechanism and the associated oxidative stress. On the other hand, a significant increase in GSH and catalase level, as well as a significant reduction in MDA level in the HIAR, treated group when compared to disease control proved the antioxidant effect of *Hemidesmus indicus* var. *pubescens*.

The colon section of disease control group showed disruption of the glandular epithelial layer and infiltration of inflammatory cells which may take the form of cryptitis, which indicates the chronicity. Animals receiving HIAR 200 mg/kg showed distorted glandular epithelium with mild inflammatory cell infiltration and crypt abscess. Moreover, the histopathology of animals receiving HIAR 400 mg/kg showed partially intact goblet cells in the epithelial layer with relatively low damage to the Crypts of Leiberkuhn and fewer crypt abscess. The standard group depicts a colon tissue with very mild glandular epithelial distortion and the presence of only a few

crypt abscesses which indicates that the standard drug has effectively treated the cellular damage caused by the induction of disease. *Hemidesmus indicus* var. *pubescens* significantly attenuated the injury caused to the mucosa and submucosal layers of the colon, suggesting that the HIAR was effective in maintaining the integrity of the colon from the oxidative stress caused by DSS. From the above results, it can be concluded that the aqueous root extract of *Hemidesmus indicus* var. *pubescens* showing remarkable changes of haematological, biochemical, UI, DAI and histopathological parameters possess anti ulcerative colitis properties. Additionally, isolation and identification of the active principle from the extract of *Hemidesmus indicus* var. *pubescens* can be done to determine the active constituents involved in the antioxidant and antiulcer properties. Further studies on the effect of pro-inflammatory cells in the development of UC can be performed. Also, the effect of the microbiota in the large intestine can be studied as it influences the immune responses in the gut.

CONCLUSION

It could be concluded that the antioxidant activity could account for the protective effect of HIAR against DSS/ethanol-induced UC. However further investigation was required to elucidate the molecular mechanism and the active principles involved in producing these effects.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

HIAR: *Hemidesmus indicus* var. *pubescens* aqueous root extract; **DSS:** Dextran Sodium Sulfate; **UC:** Ulcerative colitis; **DAI:** Disease Activity Index; **UI:** Ulcer Index; **IBD:** Inflammatory Bowel Disease; **MDA:** Malondialdehyde; **GSH:** Glutathione **TBA:** Thiobarbituric acid; **TCA:** Trichloro acetic acid; **HCl:** Hydrochloric acid; **H_2O_2:** Hydrogen peroxide.

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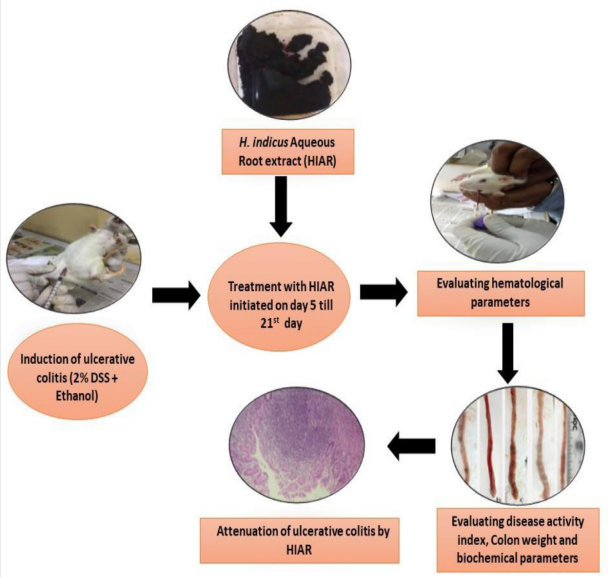
PICTORIAL ABSTRACT



SUMMARY

The present study investigated the anti-ulcerative colitis potential of *Hemidesmus indicus* var. *pubescens* aqueous root extract (HIAR) against DSS induced-ethanol augmented ulcerative colitis in rats. Anti-UC effect of HIAR was evaluated by the haematological, biochemical and histopathological parameters in colitis rats. The results showed that HIAR remarkably attenuated UC in rats, by ameliorating intestinal inflammation and reducing oxidative stress. This study confirmed the potentiality of HIAR to mitigate UC indicating its potential in the management of the same.

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