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Therapeutic Efficacy of *Ipomoea turpethum* and *Ipomoea pes-caprae* Extracts in DSS-induced Ulcerative Colitis

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Abstract

Background: Inflammatory Bowel Disease (IBD) represents chronic mucosal inflammation caused by immune dysregulation, oxidative stress, and dysfunction of the epithelial barrier, and generally includes Crohn's disease and ulcerative colitis. Current treatments have several limitations due to variable efficacy, common adverse effects, and high costs associated with their long-term use. Thus, there is an urgent need for newer and safer alternatives. Plants of Ipomoea species, viz. *I. pes-caprae* and *I. turpethum* are traditionally used medicinal plants that contain bioactive compounds, which possess antioxidant and anti-inflammatory activities, and therefore may be of therapeutic benefit in IBD. **Aim:** To evaluate the therapeutic efficacy of ethanolic extracts of *Ipomoea turpethum* (IT) and *Ipomoea pes-caprae* (IPC) in ameliorating dextran sulfate sodium (DSS)-induced ulcerative colitis. **Method:** This study used the chronic DSS-induced IBD model to conduct a 6-week study, which comparatively evaluated extracts of *I. turpenthum* and *I. pes-caprae*. Different doses of extracts were given during the study. Activity was evaluated by clinical, biochemical, and histopathological parameters. **Result:** In the carrageenan-induced paw oedema model, both extracts remarkably inhibited the inflammation at high dose to the 1.8±0.08 ml and 1.9±0.10 ml, respectively, which is comparable to the volume reduced by the standard drug diclofenac (1.7±0.10 ml). In the disease control group (DS), the body weight dropped to 185.5±6.0 g, whereas it was reversed in the IT HD treated group to 222.1 ± 6.6 g and 249±5 g in the IPC HD group. Moreover, the extract also normalised the stool consistency. Treatment with both extracts lowered the MDA from 1650 ± 60 nmol/g to 1000 ± 40 nmol/g and normalised SOD to 215 ± 15 U/g. The serum TNF-α and IL-6 levels also decreased to 980 pg/ ml and 170 pg/ml. **Conclusion:** The result obtained supports the further development of extracts for the management of inflammatory bowel disease.

Major Finding: Both extracts demonstrated therapeutic efficacy by improving weight loss of weight, improving stool consistency, and reducing rectal haemorrhage.

Keywords: Anti-inflammatory, Carrageenan, Dextran Sulfate Sodium, *Ipomoea pes-caprae*, *Ipomoea turpethum*, Ulcerative Colitis

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1. Introduction

Inflammatory Bowel Disease (IBD) comprises chronic, recurrent inflammatory conditions of the gastrointestinal tract, primarily Crohn's disease and ulcerative colitis, caused by dysregulated mucosal immunity, oxidative stress, and malfunction of the epithelial barrier. Current pharmacotherapies, such as NSAIDS, steroidal

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anti-inflammatory agents, immunomodulatory, and biologics, alleviate inflammation but are constrained by inconsistent effectiveness, unpleasant effects, and elevated costs, generating a need for complementary, safer therapeutic alternatives. IPC, also termed as costal halophyte, and IT, a traditional Ayurvedic remedy also known as Trivrit, have historically been utilised to alleviate inflammation and gastrointestinal disorders. Phytochemical analyses indicate the presence of flavonoids, terpenoids, phenolic acids, and alkaloids in these species, which are compounds that influence cytokine production, oxidative stress, and the recruitment of immune cells[1,2]. Preclinical research supports the rationale for protective mucosal uses by showing that *I. pes-caprae* extracts improve antioxidant defences, lower inflammatory markers, and promote tissue healing in cellular and animal models[3,4].

Similarly, in mouse models, hydroalcoholic and other solvent extracts of *I. turpethum* have shown protection, decreased neutrophil migration, and oedema inhibition- findings consistent with anti-inflammatory actions[5,6]. Despite these promising results, there are few direct comparisons for intestinal inflammation associated with IBD. Significant gaps include incomplete safety profiling at therapeutic doses, limited mechanistic delineation (cytokine suppression versus antioxidant buffering), and variability in extraction. Relative efficacy, mechanism, and translational potential will therefore be made clear by a methodical comparative analysis that incorporates phytochemical characterisation, supplementary pharmacological studies, and an *in vivo* colitis model. In order to guide preclinical priority and the eventual clinical translation of botanical treatment for IBD, this study makes such a comparison[5,7].

2. Materials and Methods

2.1 Materials

Fresh plant samples of *Ipomoea pes-caprae* (L.) R. Br (Figure 1) and *Ipomoea turpenthum* (L.) R. Br. (Figure 2) were collected from the coastal region and inland areas of the Daman District. The botanical identity was confirmed by the Department of Botany at Parul University, Vadodara, Gujarat, India. The authenticated herbarium specimens were prepared for future reference and documentation. The DSS 250 g (Dextran Sulphate Sodium with molecular weight 30-50 kDa) was purchased from the Sisco Research Laboratory Pvt. Ltd. (SRL), India. The sulfasalazine available with the brand name Saaz-DS tablet dosage form from the IPCA Laboratory and the diclofenac sodium available with the brand name Voveran SR 100 tablet dosage form from the Dr. Reddy's Laboratory Ltd., were procured from the local pharmacy store.

2.2 Experimental Animals

A total of 54 healthy adult female Wistar rats were selected randomly for the study and divided into nine groups containing six animals each. Rats were maintained under standardised laboratory environmental conditions in PP (Polypropylene) cages. Conditions included a 12 h light-dark cycle, room temperature at 22 ± 2°C and relative humidity of 50 ± 10%. Animals were provided with an ad libitum supply of pellet diet and clean drinking water[8-10]. Ethical approval for the study protocol was obtained from the Institutional Animal Ethical Committee (IAEC, approval number- PIPH 04/24) after thorough review. All the animal handling procedures were conducted in strict adherence to CCSEA (Committee for the Control and Supervision of Experiments on Animals) ethical guidelines to ensure humane and ethical treatment of animals.

2.3 Processing of Plant Material and Preparation of Extracts

The collected plant materials were cleaned thoroughly with tap water and followed by distilled water to remove debris and contaminants. The aerial parts of the plant were separated and dried under the shade, then powdered using a mechanical grinder and stored in airtight containers. Extract of powdered drugs was prepared by using 95% ethanol in a Soxhlet apparatus until a colourless siphonate was obtained. The obtained extract was evaporated to dryness under reduced pressure using a rotary evaporator to get dried ethanolic extracts of IT and IPC[11].

2.4 Phytochemical Screening of Plant Extracts

Phytochemical screening of both plant extracts was performed using standard qualitative methods to detect the presence of alkaloids, flavonoids, tannins, saponins, glycosides, steroids, terpenoids, and phenols[12-14].

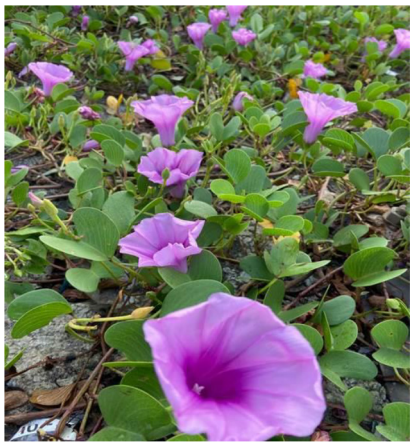


Figure 1. Image of *Ipomoea pes-caprae* Plant grown in Daman, India.



Figure 2. Image of *Ipomoea turpethum* plant grown in Daman, India.

2.5 Acute Anti-inflammatory Activity Assessed by Carrageenan-induced Paw Oedema

The carrageenan-induced hind paw oedema model was used to study the anti-inflammatory efficacy of plant extracts. 0.1 ml carrageenan was administered in the subplantar region of the right hind leg. The thickness of the paw was measured at 0 h and 24 h after the administration of carrageenan to measure the developed oedema[15,16]. 5 animal containing 7 groups were made, entitled as normal control, disease control group, low dose 50 mg/kg and high dose 500 mg/kg IT extract group and low dose 50 mg/kg and high dose 500 mg/kg IPC extract group and standard group which received diclofenac sodium 10 mg/kg dose, p.o. The % inhibition of oedema calculated for each group was estimated, which was compared with the standard drug group to ascertain the anti-inflammatory activity of plant extracts.

2.6 Effects of Extracts on DSS-Induced Ulcerative Colitis

Rats were induced with chronic ulcerative colitis through the oral administration of 5%w/v DSS in drinking water for 7 days, followed by a three-day recovery period using distilled water. This ten-day induction/recovery cycle was repeated 4 times. The consistent exposure to around 100 ml 5% w/v DSS solution was given to the three rats housed together. Six animals containing nine groups were made, and the dosing is mentioned in Table 1[17,18].

2.7 Parameters Evaluated

2.7.1 Clinical Observations (Overall Disease Activity Index: (DAI) Score)

Daily monitoring of clinical colitis indicators includes systematic recording of body weight, stool consistency, and the Disease Activity Index (DAI). The DAI index, which provides a comprehensive metric of disease severity, was calculated based on the presence of blood in stool, % of weight loss and stool consistency[19].

2.7.2 Haematological Analysis

Blood samples obtained from the retro-orbital plexus under the effect of mild anaesthesia were collected in EDTA-coated tubes. The assessment of haematological parameters was performed by using an automated haematological analyser. These parameters include RBC count, WBC count, haemoglobin concentration, and leukocyte profile[20].

2.7.3 Biochemical Assays Using Serum

Venous blood was collected from the mice and allowed to coagulate at ambient temperature for 30 min to 1 hr. The serum was isolated by centrifugation at 1000 to 2000 RPM for 15 to 20 min and stored at -20 to -80°C for further analysis. Serum levels of tumour necrosis factor-alpha (TNF-α) and Interleukin-6 (IL-6) were measured using the Krishgen Biosystem ELISA kit by following the protocol provided by the manufacturer. The sample was analysed along with standard and control in the microtiter plate by the standard incubation and washing cycles, followed

Table 1. DSS induced ulcerative colitis

Sr. No.	Groups (n=6)	Disease induction	Drug Administered
1	Control	-	Saline (p.o.)
2	Disease Control	DSS 5% for 7 days in drinking water, followed by 7 days DW (3 cycles),(p.o.)	Saline (p.o.)
3	Low Dose IT extract		50 mg/kg IT extract (p.o.)
4	Medium Dose IT extract		250 mg/kg IT extract(p.o.)
5	High Dose IT extract		500 mg/kg IT extract(p.o.)
6	Low Dose IPC extract		50 mg/kg IPC extract (p.o.)
7	Medium Dose IPC extract		250 mg/kg IPC extract (p.o.)
8	High Dose IPC extract		500 mg/kg IPC extract (p.o.)
9	Standard		Sulfasalazine 9 mg/kg (p.o.)

Table 2. Phytochemical test of *I. turpetham* and *I. pes-caprae*

Sr. No.	Phytochemical Test	*Ipomoea turpethum* (IT) extract	*Ipomoea pes-caprae* (IPC) extract
1	Terpenoids	+	+
2	Flavonoids	+	+
3	Phytosterols	+	+
4	Anthraquinone glycosides	+	+
5	Cardiac glycosides	+	+
6	Sugar	+	+
7	Alkaloids	+	+
8	Quinones	+	+
9	Phenols	+	+
10	Tannins	+	+
11	Saponins	+	+
12	Proteins and free amino acids	+	+

Note: (+) indicates presence of the respective phytochemical constituent.

by chromogenic development. The absorbance was recorded at 450 nm wavelength using an Agilent BioTek Synergy H1 microplate reader. The standard curve was plotted to determine the concentration. All the analyses were performed in triplicate to ensure the reproducibility and precision of the process[21].

2.7.4 Biochemical Assays Using Tissue Homogenate

Colon tissue homogenate was prepared and stored at -80°C before the analysis. The Thiobarbituric Acid Reactivity Substance (TBARS) method was used to determine the concentration of MDA at 532 nm wavelength. Superoxide dismutase activity was performed by measuring the ability of enzymes to scavenge superoxide radicals. So, the ability of the extract to inhibit the reduction of dyes by the enzyme was recorded by spectrophotometry method. All samples were analysed in triplicate to ensure the accuracy and reproducibility of the procedure[21].

2.7.5 Histopathological Examination

Colon tissue was fixed in 10% neutral buffered formalin, processed and embedded in paraffin. 5 µm thick section was taken from the specimen, followed by staining with eosin and hematoxylin dye. The section was examined under the light microscope to observe the alteration in mucosal lining, including epithelial injury, ulceration, depletion of goblet cells, crypt atrophy, oedema, and the invasion of inflammatory cell[22].

3. Result

3.1 Phytochemical Screening of Plant Extracts

The preliminary phytochemical evaluation of *I. turpenthum* (IT) and *I. pes-caprae* (IPC) ethanolic extracts was performed as per the standard methods[19]. Tests demonstrated the presence of secondary metabolites like terpenoids, flavonoids, phytosterols, anthraquinones, glycosides, cardiac glycosides, alkaloids, phenols, tannins, quinones and also primary metabolites like sugar, protein and free amino acids, which suggest the pharmacological potential of both plant extracts. These findings support the traditional therapeutic use of these plants. The result of the

preliminary phytochemical screening is illustrated in Table 2.

3.2 Acute Anti-inflammatory Activity Assessed by Carrageenan-induced Paw Oedema

The carrageenan-induced paw oedema model was used to evaluate the anti-inflammatory activity of IT and IPC extracts. The paw volume was measured in ml three hours after the carrageenan administration. As illustrated in Figure 3, the Normal Control (NC) group exhibits a baseline paw volume of 1.4±0.43 ml, which reflects the absence of inflammation. Conversely, the Disease Control (DC) group reached 2.7±0.21ml volume, confirming the effective induction of acute inflammation and maximal oedema response among the groups.

The IT extract demonstrated a pronounced dose-dependent anti-inflammatory effect against carrageenan-induced paw oedema. The IT LD group exhibited moderate reduction in oedema volume (2.4±0.10 ml) in comparison with the disease control group ($p < 0.01$). The effect of the extract intensifies with the increase in dose. The IT MD group showed reduction in paw volume to 1.9±0.10 ml, showing highly significant efficacy ($p < 0.001$), and the IT HD group achieved the most substantial reduction in paw volume at 1.8±0.08 ml, showing highest activity in IT extract treated groups ($p < 0.001$) very close to standard drug.

Dose-dependent anti-inflammatory activity is also noted for IPC extract. Oedema volume for the IPC LD group, IPC MD group, and IPC HD group were identified as 2.9 ± 0.12 ml ($p < 0.001$), a non-significant reduction of 2.8 ± 0.12 and 2.5 ± 0.10 ml ($p < 0.05$). IPC HD group efficacy nearly mirrored that of the efficacy of standard drugs, 1.6 ± 0.095.

The Standard Drug (STD) group recorded an inflammatory response of 1.7 ± 0.10 ml, which confirmed the potential of the standard as an anti-inflammatory agent to validate the experimental model.

Both IT and IPC extracts significantly attenuated inflammation in the carrageenan- induced paw oedema model in a dose-dependent manner. The maximal dose given in the IP HD and IPC HD demonstrated anti-inflammatory activity that is comparably aligned to the response produced by the standard drug, indicating substantial therapeutic potential of these extracts as an anti-inflammatory agent.

3.3 Effects of Extracts on DSS-induced Ulcerative Colitis

3.3.1 Clinical Parameters: Body Weight

As shown in Figure 4, in the IT extract experiment, the NC group showed a steady increase in body weight from 216.6 ± 5.7 g (week 0) to 229.0 ± 6.5 g (week 6). In contrast, the DC group showed a significant reduction in body weight from 215.0 ± 4.5 g to 185.5 ± 6.0 g at week 6 ($p < 0.01$ *vs.* NC). Treatment with IT extract significantly prevented body weight loss when compared with DC. At week 6, LD-IT animals showed 209.2 ± 5.7 g, MD-IT showed 219.7 ± 7.3 g ($p < 0.05$ *vs.* DC), and HD-IT showed 222.1 ± 6.6 g ($p < 0.01$ *vs.* DC). The Standard Drug (STD) group exhibited a marked recovery with body weight reaching 226.0 ± 4.8 g ($p < 0.01$ *vs.* DC), which was comparable to the NC group.

In the IPC extract experiment, the NC group showed a progressive increase in body weight from 247.5± 3.8 g (week 0) to 258.1 ± 3 .1 g (week 6), whereas the DC group showed a continuous decline from 248.1 ± 3.8 g to 217.1 ± 3.3 g at week 6 ($p < 0.01$ *vs.* NC). IPC extract treatment significantly attenuated DC-induced weight loss. At week 6, body weight in the IPC-LD group was 243.1 ± 5.2 g ($p < 0.05$ *vs.* DC), in the IPC-MD group

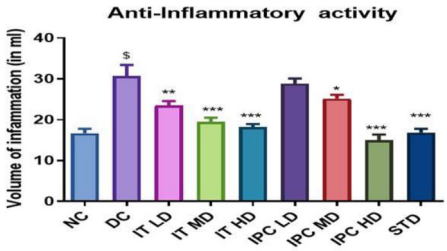


Figure 3. Anti-inflammatory activity of *Ipomoea turpethum* (IT) and *Ipomoea pes-caprae* (IPC) extracts against carrageenan-induced paw edema in Wistar rats. Values are expressed as mean ± SEM (n = 6). NC = Normal Control; DC = Disease Control; IT LD, MD, and HD = low-, medium-, and high-dose *Ipomoea turpethum* extract-treated groups; IPC LD, MD, and HD = low-, medium-, and high-dose *Ipomoea pes-caprae* extract-treated groups; STD = standard drug-treated group. Statistical significance was analyzed compared with the disease control group ($p < 0.05$, $p < 0.01$, $p < 0.001$).

246.8 ± 4.1g ($p < 0.01$ *vs.* DC), and in the IPC-HD group 248.8 ± 5.7 g ($p < 0.01$ *vs.* DC). The STD group showed a comparable recovery with a final body weight of 249 g, showing no significant difference from IPC-HD and NC groups.

Overall, comparison between IT and IPC extracts indicates that both treatments significantly improved body weight compared to DC; however, IPC extract—particularly at high dose—restored body weight more effectively and comparably to the standard drug and NC, whereas IT extract showed partial but significant recovery.

3.3.2 Stool Consistency and Rectal Bleeding Scores

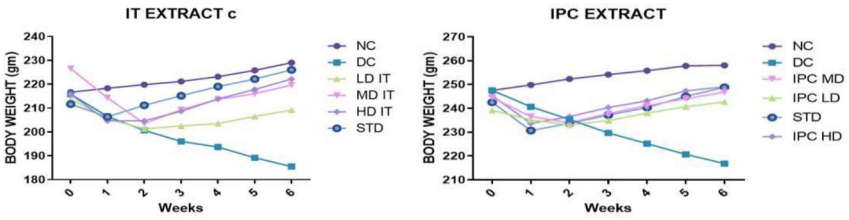
As shown in Figure 5, in the NC group, stool consistency and occult/gross rectal bleeding scores remained 0.0 ± 0.0 throughout the experimental period, indicating normal bowel function. In contrast, the DC group showed a significant worsening of both parameters, with stool consistency increasing from 2.5 ± 0.4 at week 1 to 3.3 ± 0.3 at week 2 and remaining elevated up to 1.5 ± 0.2 at week 5, while occult/gross bleeding scores increased from 1.3 ± 0.3 at week 1 to 3.3 ± 0.3 at week 4, remaining high at 2.8 ± 0.4 at week 5 ($p < 0.01$-0.001 *vs.* NC). Treatment with the standard drug (STD) significantly ameliorated disease severity, reducing stool consistency scores from 2.2 ± 0.5 (week 1) to 0.7 ± 0.2 (week 5) and bleeding scores from 1.5 ± 0.4 to 0.1 ± 0.1 ($p < 0.001$ *vs.* DC). IT extract produced a dose-dependent improvement, with week-5 stool consistency scores of 1.0 ± 0.4 (IT-LD), 0.5 ± 0.2 (IT-MD), and 0.3 ± 0.2 (IT-HD) and corresponding bleeding scores of 1.0 ± 0.2, 0.6 ± 0.3, and 0.3 ± 0.2, respectively, showing significant reductions compared to DC. IPC extract demonstrated superior efficacy, with week-5 stool consistency scores of 0.8 ± 0.3 (IPC-LD), 0.5 ± 0.3 (IPC-MD), and 0.2 ± 0.2 (IPC-HD) and bleeding scores of 0.5 ± 0.2, 0.7 ± 0.3, and 0.0 ± 0.0, respectively, where IPC-HD completely normalised both parameters, yielding values comparable to NC and the standard treatment ($p < 0.001$ *vs.* DC).

3.4 Parameters Evaluated

3.4.1 Clinical Observations (Overall Disease Activity Index (DAI) Score)

In the IT extract experiment, the normal control (NC) group showed a steady increase in body weight from 216.6 ± 5.7 g (week 0) to 229.0 ± 6.5 g (week 6). In contrast, the disease control (DC) group showed a significant reduction in body weight from 215.0 ± 4.5 g to 185.5 ± 6.0 g at week 6 ($p < 0.01$ *vs.* NC). Treatment with IT extract significantly prevented body weight loss when compared with DC. At week 6, LD-IT animals showed 209.2 ± 5.7 g, MD-IT showed 219.7 ± 7.3 g ($p < 0.05$ *vs.* DC), and HD-IT showed 222.1 ± 6.6 g ($p < 0.01$ *vs.* DC). The STD group exhibited a marked recovery with body weight reaching 226.0 ± 4.8 g ($p < 0.01$ *vs.* DC), which was comparable to the NC group.

In the IPC extract experiment, the NC group showed a progressive increase in body weight from 247.5± 3.8 g (week 0) to 258.1±3.1 g (week 6), whereas the DC



(A) (B)

Figure 4. Effect of *I. turpethum* **(A).** and *I. pes-caprae* **(B).** Extract on Body Weight in DSS-induced IBD (week 0–6) Values are expressed as mean ± SEM (n = 6). Statistical analysis was performed using one-way ANOVA followed by Tukey's post-hoc test. Disease control (DC) animals showed a significant reduction in body weight compared with the normal control (NC) group ($p < 0.001$). Treatment with IT and IPC extracts demonstrated dose-dependent improvement in body weight compared with the DSS-induced disease control group. High-dose treated groups exhibited significant restoration of body weight close to the standard-treated group. Significance levels: ($p < 0.05$), ($p < 0.01$), ($p < 0.001$) compared with disease control group.

group showed a continuous decline from 248.1± 3.8 g to 217.1±3.3 g at week 6 ($p < 0.01$ *vs.* NC). IPC extract treatment significantly attenuated DC-induced weight loss. At week 6, body weight in the IPC-LD group was 243.1±5.2 g ($p < 0.05$ *vs.* DC), in the IPC-MD group 246.8 ± 4.1g ($p < 0.01$ *vs.* DC), and in the IPC-HD group 248.8±5.7 g ($p < 0.01$ *vs.* DC). The STD group showed a comparable recovery with a final body weight of 249 g, showing no significant difference from IPC-HD and NC groups.

Overall, comparison between IT and IPC extracts indicates that both treatments significantly improved body weight compared to DC; however, IPC extract—particularly at high dose—restored body weight more effectively and comparably to the standard drug and NC, whereas IT extract showed partial but significant recovery.

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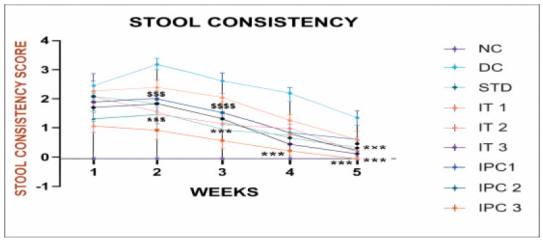


Figure 5. Effect of experimental treatments on stool consistency scores over the study period (Weeks 1–5). Data were presented in Mean ± SEM. Two-way ANOVA followed by boneferroni's test was applied to check significance level ($p<0.05$) ,*indicates the significant difference when compared to control groups, $ indicates the significant difference when compared to disease control groups.

3.5 Parameters Evaluated

3.5.1 Clinical Observations (Overall Disease Activity Index (DAI) Score)

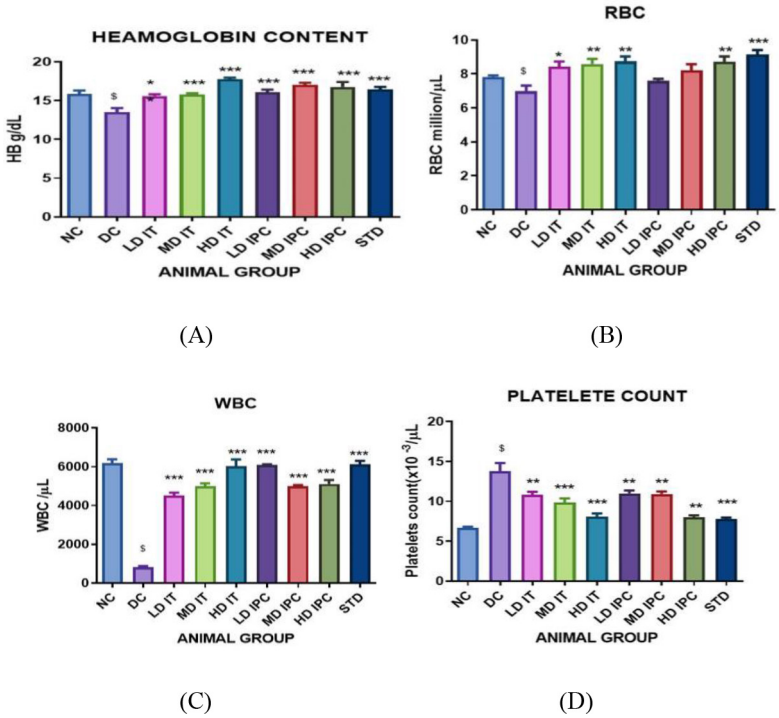
The Disease Activity Index (DAI) was calculated based on the growth reduction percentages, which demonstrate a significant and progressive development of disease control group (DC) and validate the induction of severe colitis by DSS. Specifically, the DAI of the DC group increased steadily from 8.5% in the 1st week to 29.5% by the 6th week. Whereas the normal control group (NC) was maintained at a constant negative DAI, which is -1.7 % to -12.4% over the period of time, showing healthy growth and absence of inflammatory response.

By the 4th week, the DAI in the DC group reached 21.3%. However, the HD IT, LD IPC, and HD IPC group showed a drastic reduction in score of 1.9%, 1.2% and 2.2%, respectively. This difference escalated by the 6th week. While the DC group reached 29.5%, the HD IT at -6.4%, HD IPC at -4.1% and STD at -6.3%, which represent robust recovery, characterised by the recovery from weight loss.

At the 6th week, HD IT came up as an effective IT treatment, surpassing LD IT and MD IT groups at 4.6% and 6.8%, respectively. The IPC treatment exhibited a dose-responsive trend with DAI -4.1% and achieved greater symptom reduction than the lower dose counterparts. Collectively, both extract treatments have significantly lowered the DAI score by dose and time contingent. The comparative advantage of high doses of IT and IPC over the standard drug suggests the promising significance of their utility in the management of colitic inflammation.

3.5.2 Hematological Parameters

As illustrated in Figures 6, DSS administration produced marked haematological alterations in the DC group compared with the NC. Haemoglobin (Hb) levels were significantly reduced in the DC group (13.2 g/dL) compared to the NC (15.8 g/dL), indicating DSS-induced anaemia. Treatment with *I. turpethum* (IT) and *I. pes-caprae* (IPC) extracts significantly restored Hb levels in a dose-dependent manner, with medium- and high-dose groups showing values ranging from 15.6 to

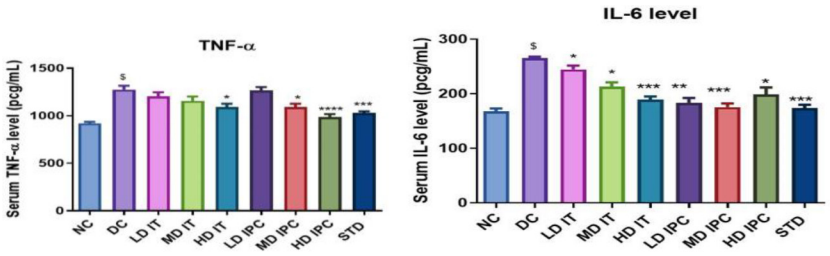


(A) (B)

(C) (D)

Statistical symbols: data are expressed as mean ± SEM (n = 6). *p < 0.01, ***p < 0.001 vs. DC $ p < 0.001 *vs*. NC. Data were presented in Mean ± SEM. One-way ANOVA followed by Tukey's test was applied to check significance level (p < 0.05);* indicates the significant difference when compared to control groups, $ indicates the significant difference when compared to disease control groups.

Figure 6. **(A).** Effect of different treatments on haemoglobin (Hb) content across experimental animal groups. **(B).** Effect of different treatments on red blood cell (RBC) count in experimental animal groups. **(C).** Effect of different treatments on white blood cell (WBC) count in experimental animal groups. **(D).** Effect of different treatments on platelet count in experimental animal groups.



(A) (B)

Data were presented in Mean ± SEM. One-way ANOVA followed by Tukey's test was applied to check significance level (p < 0.05). * indicates the significant difference when compared to control groups, $ indicates the significant difference when compared to disease control groups.

Figure 7. **(A).** Effect of different treatments on serum tumour necrosis factor-α (TNF-α) levels in experimental animal groups. **(B).** Effect of different treatments on serum interleukin-6 (IL-6) levels in experimental animal groups.

17.5 g/dL ($p < 0.001$), comparable to the STD group. Similarly, RBC count was significantly decreased in the DC group (6.9×10^6 cells/µL) compared with NC (7.8×10^6 cells/µL). Administration of medium and high doses of IT and IPC significantly increased RBC counts ($8.4\text{-}9.0 \times 10^6$ cells/µL; $p < 0.01\text{-}0.001$), with high-dose IPC and STD groups exhibiting maximal recovery. A marked reduction in WBC count was observed in the DC group (750 cells/µL) relative to NC (6100 cells/µL), indicating DSS-induced immunosuppression. Treatment with IT and IPC significantly restored WBC counts, particularly in high-dose IT, medium-dose IPC, and high-dose IPC groups (**5000-6200 cells/µL; $p < 0.001$). In contrast, platelet count was significantly elevated in the DC group, reflecting DSS-induced inflammatory thrombocytosis. Treatment with medium and high doses of IT and IPC significantly reduced platelet counts ($p < 0.001$), with high-dose IPC normalising values comparable to the STD group. Collectively, these findings demonstrate that IT and IPC extracts effectively ameliorate DSS-induced haematological abnormalities through haemato-protective, immunomodulatory, and anti-inflammatory actions.

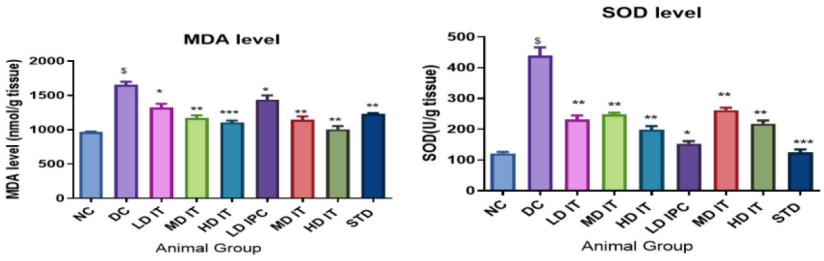
3.5.3 Biochemical Assays using Serum- TNF-α Levels and IL-6 Levels

The DC group exhibited a marked elevation in pro-inflammatory cytokines, demonstrating DSS-induced inflammation. Serum TNF-α levels increased significantly in the DC group (1250 pg/mL) compared with the NC 920 pg/mL; $\$p < 0.05$). As shown in Figure 7. Treatment with IT and IPC extracts produced a dose-dependent suppression of TNF-α. Low- and medium-dose IT reduced TNF-α to 1180 and 1140 pg/mL, respectively, while high-dose IT further lowered the level to 1080 pg/mL (*$p < 0.05$ *vs.* DC). Among IPC-treated groups, medium-dose IPC reduced TNF-α to 1100 pg/mL (*$p < 0.05$), whereas high-dose IPC produced the most pronounced reduction (980 pg/mL; ****$p < 0.0001$), comparable to the standard drug group (1020 pg/mL; ***$p < 0.001$).

Similarly, serum IL-6 levels were significantly elevated in the DC group (265 pg/mL) relative to NC animals (165 pg/mL; $\$p < 0.05$). Administration of IT and IPC extracts significantly attenuated IL-6 levels across doses. Medium-dose IT reduced IL-6 to 215 pg/mL (*$p < 0.05$), while high-dose IT showed a stronger effect (190 pg/mL; ***$p < 0.001$). IPC treatment demonstrated notable efficacy, with medium-dose IPC reducing IL-6 to 175 pg/mL (***$p < 0.001$) and high-dose IPC to 200 pg/mL (*$p < 0.05$). The standard drug group showed a significant reduction (170 pg/mL; ***$p < 0.001$). Overall, these findings indicate that IT and IPC effectively suppress DSS-induced TNF-α and IL-6-mediated inflammatory responses. Statistical symbols: $\$ p < 0.05$ *vs.* NC; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ *vs.* DC.

3.5.4 Colon Tissue Homogenate Analysis- MDA Levels and SOD Levels

The effect of IT and IPC treatment on oxidative stress in colonic tissue is illustrated in Figure 8. In the DC group, the level of Malondialdehyde (MDA)



(A) (B)

Data were presented in Mean ± SEM. One-way ANOVA followed by Tukey's test was applied to check significance level ($p < 0.05$); *indicates the significant difference when compared to control groups, $ indicates the significant difference when compared to disease control groups.

Figure 8. **(A).** Effect of different treatments on Malondialdehyde (MDA) levels in colonic tissue of experimental animal groups, **(B).** Effect of different treatments on Superoxide Dismutase (SOD) levels in colonic tissue of experimental animal groups.

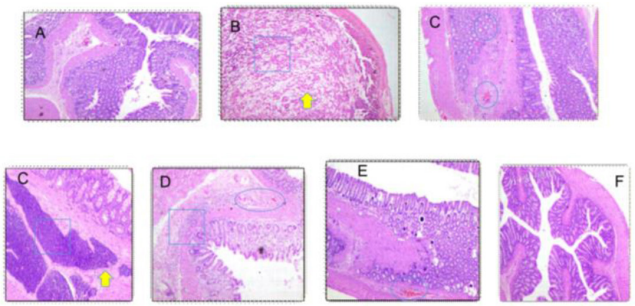


Figure 9. Representative H and E-stained (40×) colonic sections. **(A).** NC: normal mucosal lining and glandular architecture. **(B).** DC: severe epithelial degeneration, leukocyte infiltration, ulceration, and glandular damage. **(C).** STD: marked restoration of colonic architecture with reduced inflammation. **(D).** IPC-LD: partial epithelial protection and moderate inflammatory changes. **(E).** IPC-HD: near-normal histology with minimal leukocyte infiltration. **(F).** IT-LD: moderate reduction in inflammation and glandular degeneration. **(G).** IT-HD: pronounced protection with restored glands and minimal inflammatory changes. Black arrows indicate leukocyte infiltration; red arrows indicate epithelial/glandular degeneration; dashed arrows indicate ulcerated regions.

is remarkably increased (1650 ± 60 nmol/ g tissue) as compared to the NC group (980±40 nmol/g tissue), which indicates an increase in lipid peroxidation. But the treatment with IT and IPC extract reduced the MDA level in a dose-dependent manner. IT MD and IT HD showed a decrease in MDA level to 1150±45 nmol/g tissue and 1080±40 nmol/g tissue, respectively. On the other hand, IPC HD showed a remarkable reduction, 1000±40 nmol/g tissue. The standard drug treatment also showed a noteworthy reduction in MDA level (1200±45 nmol/g tissue).

In the DC group, Superoxide Dismutase (SOD) activity was notably higher than the NC group, which is 440 ± 20 U/g and 120 ± 8 U/g, respectively. The treatment with IP and IPC extract remarkably reduced the SOD activity at all the doses. Most pronounced antioxidant activity was observed in IT HD and IPC HD, which is 200 ± 14 U/g and 215 ± 15 U/g, respectively. On the other hand, treatment with the standard drug brought the activity of SOD very close to the normal level, which is 102 ± 10U/g. Overall, all assays show that the IT and IPC extracts effectively managed the DSS-induced oxidative stress by reducing lipid peroxidation.

3.5.5 Histopathology of Colon

Histopathological examination of Hematoxylin and eosin-stained colon sections under 40 x magnification revealed (Figure 9) the dose-dependent protective effect. The NC group exhibits the intact mucosal layer characterised by the straight, parallel crypts and no inflammation. Whereas the DC group was characterised by the severely damaged epithelial layer, infiltration of leukocytes, mucosal ulceration, and glandular atrophy. The IPC LD group showed moderate damage to the colon tissue with less inflammation than the DS group; on the other hand, the IPC HD group exhibited improvement with reduced leucocyte infiltration and partial recovery of mucosal lining. The IT LD treatment group showed only mild damage to the epithelial layer and marginal improvement in glandular damage. The IT HD and standard drug treatment group showed nearly normal histology of colon tissue, with an intact mucosal layer, minimal inflammation, and restored glandular structure. Hence, the treatment with both extracts, especially at higher doses, effectively mitigated the colonic tissue destruction and inflammation.

4. Discussion

Phytochemical screening of IT and IPC extracts revealed diverse bioactive metabolites, including terpenoids, flavonoids, phytosterols, anthraquinone glycosides, and cardiac glycosides. Both extracts at high dose showed remarkable dose-dependent anti-inflammatory response against the carrageenan-induced paw oedema model, comparable to the standard drug.

In the DSS-induced ulcerative colitis model, IT extract recovered the weight loss and improved the stool consistency in the dose dependent mane. Whereas IPC managed the condition very close to that of the standard drug response. Also countered the DSS induced declined hemoglobin, RBC, and WBC counts, and normalised the elevated platelet numbers, which proves the hematoprotective and immunomodulatory effect of extracts.

IT and IPC extract remarkably reduced pro-inflammatory cytokines TNF-α and IL-6. Treatment with IPC HD demonstrates the strongest suppression response, comparable to the standard drug. This treatment also normalised the response of oxidative

stress markers like MDA level and superoxide dismutase activity, which represents the antioxidant effect.

Histopathological data reflect the dose-dependent mitigation of DSS-induced colonic damage. At high doses, IT and IPC extracts restore the mucosal lining and reduce inflammation. This finding supports the claim that IT and IPC extracts have anti-inflammatory, antioxidant, hematoprotective, and immunomodulatory potential.

5. Conclusion

The study concludes that *I. turpenthum* (IT) and *I. pes-caprae* (IPC) extracts contain diverse bioactive phytochemicals that contribute to their significant anti-inflammatory, antioxidant, immunomodulatory, and hematoprotective effects. Both extracts demonstrated dose-dependent efficacy in reducing inflammation, oxidative stress, and haematological disturbances in the DSS-induced ulcerative colitis model, with high doses showing effects comparable to standard treatment. Histopathological improvements further confirmed their capacity to restore colonic tissue integrity and reduce inflammation. These findings support the potential therapeutic application of IT and IPC extracts as effective agents for managing inflammatory bowel diseases.

From a translational perspective, further research should focus on detailed mechanistic delineation, including effects on specific immune signalling cascades, epithelial-barrier proteins, and gut microbiota modulation. Standardisation of extraction procedures, identification of active marker compounds, and dose-range safety profiling are essential next steps. Future work incorporating pharmacokinetic evaluation, chronic toxicity studies, and combination approaches with existing drugs could clarify preclinical priority. Ultimately, well-designed clinical investigations will be required to establish efficacy and safety in human subjects and to advance development of affordable plant-based therapeutics for IBD management.

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Author Contribution

DT and AN carried out the data collection and analysis in addition to contributing to the study design and conceptualisation. The results' statistical analysis and interpretation fell under the purview of ABS. In addition to drafting the first manuscript, NP and HNP helped with the methodology and experimental design. Data interpretation and text changes were aided by AN, HNP, and DT. The manuscript was edited and completed for submission by ABS and NP. All authors have read and agreed to the published version of the manuscript.