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Persicaria lanigera (Polygonaceae) leaf extract exhibits antiulcerogenic and antiproliferative activities against acetic acid-induced ulcerative colitis and cotton pellet-induced granuloma tissue in rats

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ABSTRACT

Objective: To assess the effect of leaf extract of *Persicaria lanigera* on cotton pellet-induced granuloma tissue formation and acetic acid-induced ulcerative colitis.

Methods: Rats were randomly divided into six groups: normal control, negative control, positive control (dexamethasone or sulfasalazine) as well as *Persicaria lanigera* (100-600 mg/kg)-treated groups. The effects of the extracts on body weight, antioxidant, and hematological parameters, as well as mast cell proliferation, were assessed. In addition, a histological evaluation was conducted.

Results: *Persicaria lanigera* extract significantly decreased the mean exudate amount and suppressed granuloma tissue formation in a concentration-dependent manner in rats ($P < 0.05$). Additionally, the extract significantly increased body weight, improved hematological profile, reduced the disease activity index score and malondialdehyde level, as well as enhanced catalase and superoxide dismutase activities ($P < 0.05$). Histological evaluation showed *Persicaria lanigera* extract alleviated acetic acid-induced colonic damages, as evidenced by decreased cell necrosis, edema, and inflammatory cell infiltration.

Conclusions: *Persicaria lanigera* extract possesses antiproliferative, antioxidative, and anti-colitis activities. However, its underlying mechanisms of action need further investigation.

KEYWORDS: Colitis; *Persicaria lanigera*; Antioxidant; Cotton pellets; Mast cells; Granuloma tissue formation

1. Introduction

The world is plagued with numerous chronic inflammatory disorders that affect every system, organ, and tissue in the body, causing varying degrees of structural and functional alterations depending on the severity and duration of the inflammation. Such modifications reduce the quality of life and frequently necessitate lifetime medical care, two major outcomes that are seriously detrimental to societal and financial effects[1].

Significance

We have previously reported that *Persicaria lanigera* possesses anti-inflammatory activities; however, its effects on digestive disorders and cell proliferation have not been reported. Our studies demonstrated that *Persicaria lanigera* leaf extract inhibited cotton pellet-induced granuloma tissue formation and ameliorated acetic acid-induced ulcerative colitis.

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Inflammation plays a pivotal role in numerous medical disorders[2]. The prevalence of illnesses such as inflammatory bowel diseases, ischaemic heart disease, asthma, ulcers, atherosclerosis, rheumatoid arthritis, cancer, *etc.* caused by irritants, infections (pathogens) due to deregulated acute inflammation, and other unwanted immune reactions are high in many people from developing countries[3]. Most drugs such as non-steroidal anti-inflammatory drugs, steroids, disease-modifying anti-rheumatic drugs, and other biologics are utilized currently to manage inflammation. However, the use of these agents is believed to be connected with a slew of possibly life-threatening undesirable consequences such as gastrointestinal tract disorders, skin rashes, dyspepsia, obesity, *etc.*[4]. Consequently, it is imperative to look for newer and more diverse sources of medicinal substances with potential anti-inflammatory activities to treat chronic inflammation as well as other associated conditions.

Herbal medicine has been the dominant traditional form of health care assessed by humans. Today, in about 80% of the world's population, plant medicine has been developed to become the primary source of medicine, and many people resort to herbal therapy[5]. The discovery of new pharmacological agents may benefit from the use of novel compounds found in plants, and it is believed that about a quarter of all prescription pharmaceuticals worldwide originate from plant sources[6].

Typically, certain plant parts including leaves, roots, flowers, fruits, stems, and seeds are employed in herbal formulations or as pure active ingredients that are then transformed into beneficial treatments. Solid scientific information about the biological actions of several herbal remedies utilized in traditional medicine is available[7].

Many medical benefits have been attributed to the *Persicaria* species, which contains an abundance of phytochemical components including anthraquinones, flavonoids, and terpenoids. They have traditionally been employed as remedies to alleviate a variety of pain disorders[7]. *Persicaria lanigera* (*P. lanigera*) is a very common plant in Ghana that is typically used to alleviate some pain as well as other inflammatory conditions[7]. Recently, we have demonstrated its significant effect in acute inflammation[8]. In this study, we tried to look into the actions of hydroethanolic leaf extract of *P. lanigera* on cotton pellet-induced granuloma formation and acetic acid-induced ulcerative colitis in Sprague-Dawley rats.

2. Materials and methods

2.1. Chemicals and reagents

Sulfasalazine tablets (MP-Biomedicals, California, USA), ethanol (Ernest Chemist, Accra, Ghana), dexamethasone tablets (Pharm-Inter, Brussels, Belgium), cotton wool (Ashgin Pharmacy, Cape Coast, Ghana), acetic acid (Santa Cruz Biotechnology Inc., Dallas,

Texas, USA), formaldehyde (Ernest Chemist, Accra, Ghana), superoxide dismutase (SOD), catalase and malondialdehyde (MDA) (Sigma-Aldrich, Louis, MO, USA) were utilized in the study.

2.2. Plant collection and extraction

P. lanigera fresh leaf collection, authentication, and subsequent comparison to voucher specimen (no. MAA003) in the School of Biological Sciences Herbarium at the University of Cape Coast, have been previously described[8]. The extraction conducted was previously described by Obese *et al.*[7]. *P. lanigera* extract was freshly reconstituted in 2% tragacanth mucilage (dissolved in normal saline) before administration.

2.3. Animals

The Sprague Dawley rats (150-200 g) of both genders were kept in the animal facility at the Department of Biomedical Sciences after being purchased from the Centre for Plant Medicine Research in Mampong-Akwapim, Ghana. The animals were randomly selected, kept in stainless steel cages with softwood shavings as bedding, and fed a typical commercial pellet diet obtained from Agricare, Ghana. Water was available to all animals without restriction. The animals were given enough time to become used to their new environment and were maintained at a room temperature of (25±2) °C on a 12-hour light-dark cycle with controlled conditions of humidity.

2.4. Cotton pellet-induced granuloma tissue formation

The antiproliferative effect of *P. lanigera* extract on cotton pellet-induced granuloma tissue in rats was tested using a technique first outlined by Mohankumar *et al.*[9]. In brief, rats (150-200 g, *n*=5) were separated into six groups and fasted for 8 h before the experiment with only water administered *ad libitum*. Under light ether, the rats were anesthetized with pentobarbitone (20 mg/kg, *i.p.*), and the abdomen was shaved after which the area was disinfected with 70% ethanol. At the groin region, sterilized pellets of cotton [(40±1) mg] were inserted subcutaneously on each side of the abdomen bilaterally and closed with sutures. Group I (sham) was given saline (1 mL, *p.o.*, daily); Group II (negative control) was given saline (1 mL, *p.o.*, daily); Group III was given dexamethasone (3 mg/kg, *p.o.*, daily); Group IV-VI were given *P. lanigera* extract (100-600 mg/kg, *p.o.*, daily) (doses were selected based on earlier studies by Antwi-Adjei *et al.*) [8] accordingly. Animals were monitored for 7 d and were humanely sacrificed on day 8.

The pellets were carefully retrieved (together with granular tissue that had been liberated of immaterial tissue) and promptly weighed for wet weights. The pellets were incubated at 60 °C for 24 h to achieve a consistent weight.

2.4.1. Change in body weight

The rats were weighed daily for 8 d. The percentage change in body weight of rats was determined using the equation:

$$\% \text{ change in body weight} = [(W_d - W_0)/W_0] \times 100$$

Where W_0 is the initial body weight of rats (*i.e.* day zero), W_d is the final body weight of rats (on various days).

2.4.2. Measurement of spleen weight

The weight of the spleen from individual rats in each group was weighed and recorded. The change in spleen weight for each animal was calculated by dividing the spleen weight by the absolute body weight of each rat in each group.

2.4.3. Hematological assessment

The rats were sacrificed by cervical dislocation, and blood samples were obtained into EDTA tubes *via* cardiac puncture. Using an automated Haematology Cell Diagnosis analyzer machine (OEM-JL220102001, Nanjing Jalead International Trading Co., Ltd, Nanjing, Jiangsu, China), full blood count analysis was performed.

2.4.4. In vivo antioxidant activity

Blood samples were put into vacuum separator tubes and spun in a centrifuge at $3000 \times g$ at 5°C for 15 min. The sera obtained were kept in Eppendorf tubes and stored at -80°C until use. The sera samples were assessed for catalase[10], SOD[10], and MDA[10].

2.5. Acetic acid-induced ulcerative colitis in rats

2.5.1. Induction of colitis

Colitis was caused by intrarectally injecting 1 mL of 4% acetic acid using a sterilized pediatric catheter (size: 6Ch/Fr) (SAFER-Shanghai SNWI Medical Co. Ltd, Shanghai, China) under a low dose of ether anesthesia[11,12]. Briefly, six groups of rats (150–200 g, $n=5$) were arbitrarily chosen. For 8 d, Group I (control) received saline (0.9% NaCl) orally; Group II was given 1 mL each of 4% acetic acid intrarectally and saline (orally); Group III received sulfasalazine (500 mg/kg, *p.o.*) before intrarectal injection of 1 mL of 4% acetic acid on day 4; Groups IV–VI were treated with *P. lanigera* extract (100–600 mg/kg, *p.o.*) daily and then intrarectal injection of 1 mL of 4% acetic acid into the colons of each animal in the groups on day 4.

2.5.2. Change in body weight

Changes in the weight of the test animals were monitored during the observation period. The change in body weight of rats after treatment with *P. lanigera* extract was also calculated.

2.5.3. Macroscopic ulcerative colonic damage and disease activity index (DAI) assessment

The colons from each animal were removed, cleaned, washed, and

assessed for stool consistency, weight, and length ratio (taken from 1 cm beyond the anus to the tip of the cecum) as well as macroscopic appearance. Using the Kimball *et al.*[13] scoring approach, damage to the colon was evaluated by calculating DAI score, representing the total of the values derived from the indices (Supplementary Table).

2.5.4. Hematological analysis

The rats were humanely sacrificed by cervical dislocation, and blood samples were obtained into EDTA tubes through heart puncture. A digitized Haematology Cell Diagnosis analyzer machine (OEM-JL220102001, Nanjing Jalead International Trading Co., Ltd, Nanjing, Jiangsu, China) was used to perform the whole blood count analysis.

2.5.5. Histopathological studies

Distal colons (1 cm) from the animals were removed, cleaned, and washed thoroughly. Prepared 5 μm -thick sections were dyed with hematoxylin and eosin stain, and histopathological alterations were checked using a light microscope (BUM260-Biological Upright Clinical microscope, BIOIMAGER Inc., Maple, Canada).

2.5.6. Mast cell proliferation

Carnoy's fixative was used to fix thick-segmented sections, which were then dyed with 1% toluidine blue. A micrometer grid (0.042 mm^2) was used to record mast cells in coded areas at $\times 20$ magnification. Ten adjacent mucosal sections that do not overlap beyond the muscularis mucosae were assessed for each rat.

2.5.7. Determination of oxidative stress

Colon tissues collected were cleaned, washed thoroughly, and homogenized on ice using a homogenizer (BSL Pre-PROC homogenizer, BioBase Biozone Co. Ltd, Shandong, China). The obtained homogenate was used to measure enzymatic activities of the extract in the following: catalase, SOD and MDA using a method earlier described by Yousra *et al.*[11]. The antioxidant activities were determined spectrophotometrically at respective absorbance for each biomarker.

2.6. Statistical analysis

All the results were stated as mean $\pm$ SEM ($n=5$). Data was plotted using GraphPad Prism version 8 for Windows (GraphPad, San Diego, USA) and analyzed with one-way as well as two-way ANOVA (analysis of variance). Values were considered statistically significant at $P<0.05$ following Dunnett's *post hoc* test.

2.7. Ethical statement

Throughout the experimental period, all animals utilized in this

investigation were treated humanely in accordance with the Public Health Service Policy on Humane Care and Use of Laboratory Animals. Furthermore, the rat investigations were carried out with the agreement of the UCC School of Pharmacy Research and Ethics Committee (UCCSoPPS/REC/21/018).

3. Results

3.1. Effect of *P. lanigera* extract in rats with granuloma tissue formation

3.1.1. Effect of *P. lanigera* extract on granuloma tissue formation

The antiproliferative action of *P. lanigera* extract on granuloma tissue formation induced by the cotton pellets was studied. The mean exudate amount of the negative control group was (835.30±35.38)

mg (Table 1). *P. lanigera* extract markedly decreased the mean exudate amount in a concentration-dependent manner compared to the negative control group. Moreover, the granuloma tissue formed in the control group was (132.70±7.51) mg (Table 1). *P. lanigera* extract notably inhibited granuloma tissue formation at 100, 300, and 600 mg/kg (Table 1).

3.1.2. Effect of *P. lanigera* extract on body weight

As shown in Figure 1, body weight of the negative control group was remarkably decreased significantly compared to the sham group ($P<0.01$). However, *P. lanigera* extract significantly increased body weight in a dose-dependent manner compared with the negative group ($P<0.05$).

3.1.3. Effect of *P. lanigera* extract on hematological parameters

The negative control group showed high levels of white blood

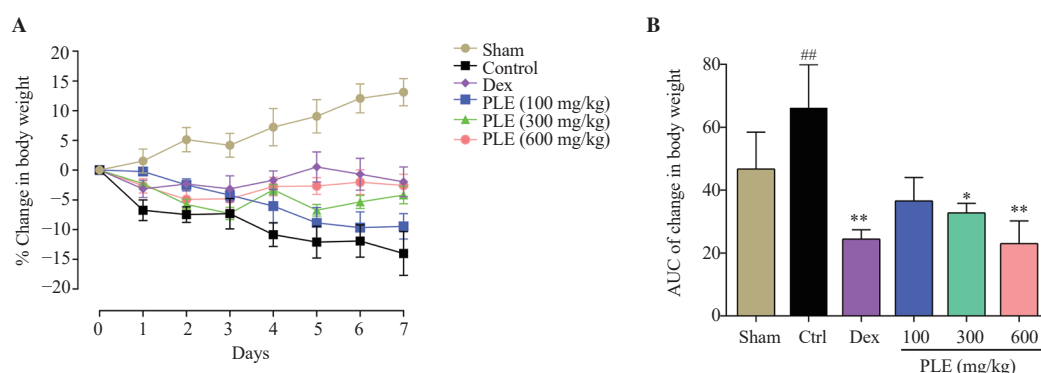


Figure 1. Effect of *Persicaria lanigera* extract on body weight of rats in cotton pellet-induced granuloma tissue formation. Values are presented as mean ± SEM ($n=5$) and analyzed by one-way or two-way ANOVA followed by Dunnet's *post hoc* test. $^{##}P<0.01$ compared with the sham group; $^{*}P<0.05$, $^{**}P<0.01$ compared with the negative control group. Dex: dexamethasone; AUC: area under curve.

Table 1. Effect of *Persicaria lanigera* extract on granuloma tissue formation in rats.

Groups	Exudate (wet weight)/mg	% Inhibition	Granuloma tissue (dry weight)/mg	% Inhibition
Negative control	835.30 ± 35.38	-	132.70 ± 7.51	-
PLE (100 mg/kg)	572.70 ± 31.34 **	31.44%	105.30 ± 4.18 *	20.65%
PLE (300 mg/kg)	496.30 ± 65.90 **	40.58%	102.70 ± 7.22 *	22.61%
PLE (600 mg/kg)	446.00 ± 60.85 **	46.61%	92.67 ± 4.91 **	30.17%

Values are presented as mean ± SEM ($n=5$) and analyzed by one-way ANOVA followed by Dunnet's *post hoc* test. $^{*}P<0.05$, $^{**}P<0.01$ compared to the negative control. PLE: *Persicaria lanigera* extract.

Table 2. Effect of *Persicaria lanigera* extract on hematological parameters in rats with cotton pellet-induced granuloma tissue.

Groups	WBC ($10^3/\mu\text{L}$)	RBC ($10^3/\mu\text{L}$)	HGB (g/dL)	PLT ($10^3/\mu\text{L}$)	MCV (fL)	NEU (%)	LMP (%)
Sham	7.66 ± 0.61	7.86 ± 0.81	14.21 ± 1.19	557.0 ± 44.0	58.45 ± 2.05	21.90 ± 1.89	45.60 ± 2.90
Negative control	16.73 ± 0.68 $^{#}$	5.66 ± 0.36	11.44 ± 1.16	896.0 ± 24.0 $^{##}$	47.64 ± 2.44	51.64 ± 5.89 $^{##}$	54.51 ± 3.75
PLE (100 mg/kg)	10.13 ± 1.89	6.54 ± 0.35	13.66 ± 0.66	729.0 ± 60.0	56.56 ± 3.26	34.99 ± 1.91 *	47.32 ± 2.78
PLE (300 mg/kg)	9.79 ± 2.02 *	7.24 ± 0.58	13.35 ± 1.05	702.5 ± 10.5	52.57 ± 2.55	31.09 ± 3.55 *	41.69 ± 4.25
PLE (600 mg/kg)	9.09 ± 1.91 *	6.94 ± 0.61	13.94 ± 0.87	673.0 ± 51.0 *	55.02 ± 4.87	28.22 ± 3.10 **	39.78 ± 2.22

Values are presented as mean ± SEM ($n=5$) and analyzed by one-way ANOVA followed by Dunnet's *post hoc* test. $^{#}P<0.05$, $^{##}P<0.01$ compared with the sham group; $^{*}P<0.05$, $^{**}P<0.01$ compared to the negative control. WBC: white blood cell; RBC: red blood cell; HGB: haemoglobin; PLT: platelet; MCV: mean corpuscular volume; NEU: neutrophil; LMP: lymphocyte.

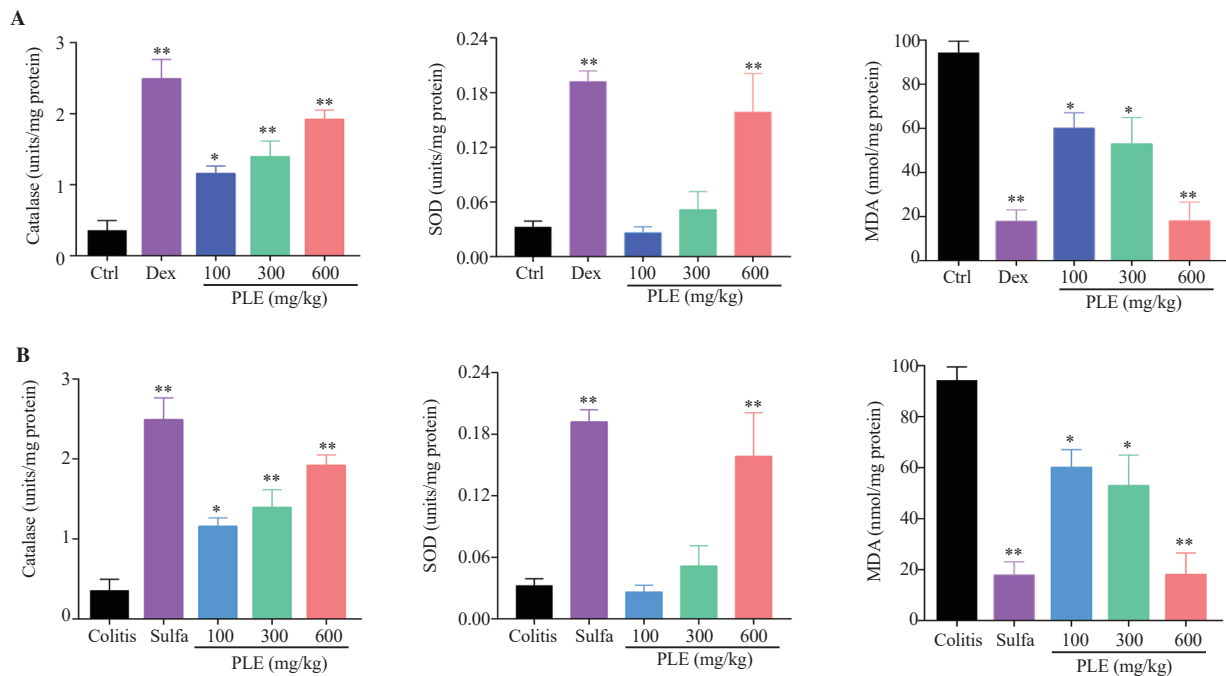


Figure 2. Effect of *Persicaria lanigera* extract on lipid oxidation and antioxidant activity in rats with (A) cotton pellet-induced granuloma tissue and (B) acetic acid-induced ulcerative colitis. Values are presented as mean $\pm$ SEM ($n=5$) and analyzed by one-way ANOVA followed by Dunnet's *post hoc* test. * $P<0.05$, ** $P<0.01$ compared with the negative control group. Sulfa: sulfasalazine.

cell (WBC), platelet (PLT), and neutrophil (NEU) ($P<0.05$) (Table 2). However, *P. lanigera* extract substantially reduced the levels of WBC at 300 mg/kg and 600 mg/kg ($P<0.05$), PLT at 600 mg/kg ($P<0.05$), and NEU at all dosages ($P<0.05$, $P<0.01$), respectively compared to the negative control group (Table 2).

3.1.4. Effect of *P. lanigera* extract on spleen weight

In all treatment groups, the spleen weight/rats' body weight was determined and recorded. There was a rise in the spleen weight to body weight ratio in the negative control group (0.68 ± 0.05) compared with the sham group (0.21 ± 0.03). *P. lanigera* extract decreased spleen weight to body weight ratio concentration-dependently (0.57 ± 0.03 , 0.53 ± 0.06 and 0.39 ± 0.07 at 100, 300, and 600 mg/kg, respectively), with 600 mg/kg showing a more significant effect.

3.1.5. Effect of *P. lanigera* extract on oxidative stress

Catalase and SOD activities in the sera of negative control rats were significantly reduced (Figure 2A). However, *P. lanigera* extract treatment increased these enzymatic activities in a concentration-dependent manner, with 600 mg/kg showing the best effects ($P<0.01$). Moreover, a high MDA level was found in the negative control group, which was reversed by treatment with all concentrations of the extract ($P<0.05$) (Figure 2A).

3.2. Effect of *P. lanigera* extract in rats with acetic acid-induced ulcerative colitis

3.2.1. Change in body weight

The negative control group demonstrated a substantial decline in rats' body weight throughout the study, with a considerable overall loss in body weight compared to the normal control group ($P<0.01$) (Figure 3). *P. lanigera* extract treatment increased body weight at 600 mg/kg ($P<0.05$) (Figure 3) compared with the negative control group.

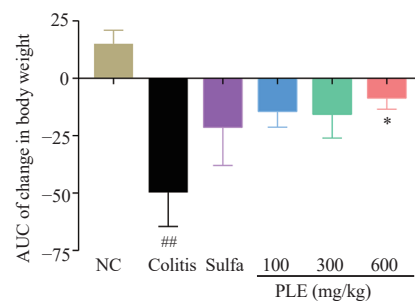


Figure 3. Effect of *Persicaria lanigera* extract on body weight of rats with acetic acid-induced colitis. Values are presented as mean $\pm$ SEM ($n=5$) and analyzed by one-way ANOVA followed by Dunnet's *post hoc* test. ## $P<0.01$ compared with the normal control (NC). * $P<0.05$ compared with the colitis control group.

3.2.2. Effect of *P. lanigera* extract on DAI and colon weight to colon length ratio

The negative control group had an extremely severe colonic injury, as evidenced by a significantly high DAI (84.00 ± 5.09 vs. 2.00 ± 1.40 , $P < 0.01$) compared to the normal control group (Figure 4A). *P. lanigera* extract treatment showed a substantial improvement in colonic injury with decreased DAI at all concentrations ($P < 0.05$) (Figure 4A).

The colon weight to colon length ratio was notably increased

($P < 0.01$) in the negative control group (Figure 4B). In contrast, *P. lanigera* extract treatment markedly lowered the colon weight to length ratio from 0.67 ± 0.05 to 0.32 ± 0.10 and 0.25 ± 0.11 at 300 mg/kg ($P < 0.05$) and 600 mg/kg ($P < 0.01$) (Figure 4B).

3.2.3. Effect of *P. lanigera* extract on mast cell proliferation

The normal control group had no enhanced inflow of mast cells to the site of injury (4.36 ± 0.89). The negative control group showed markedly increased mast cells ($P < 0.01$) compared with the normal

Table 3. Effect of *Persicaria lanigera* extract on hematological parameters in rats with acetic acid-induced ulcerative colitis.

Groups	WBC ($10^3/\mu\text{L}$)	HGB (g/dL)	RBC ($10^3/\mu\text{L}$)	PLT ($10^3/\mu\text{L}$)	LMP ($10^3/\mu\text{L}$)	HCT (%)	NEU ($10^3/\mu\text{L}$)
Normal control	4.19 ± 0.99	11.34 ± 1.03	6.44 ± 0.55	485.60 ± 88.21	3.44 ± 0.39	43.54 ± 3.89	0.59 ± 0.22
Colitis control	$10.82 \pm 1.32^{##}$	11.93 ± 0.82	5.39 ± 0.67	572.80 ± 147.70	5.11 ± 0.71	43.94 ± 3.34	$2.52 \pm 0.88^{##}$
PLE (100 mg/kg)	$5.99 \pm 1.02^*$	11.60 ± 0.67	5.88 ± 0.44	388.00 ± 116.30	2.93 ± 1.09	39.48 ± 2.49	1.71 ± 0.37
PLE (300 mg/kg)	$5.38 \pm 1.43^*$	12.02 ± 0.92	6.13 ± 0.48	471.20 ± 152.50	$2.43 \pm 0.52^*$	41.38 ± 3.23	1.38 ± 0.46
PLE (600 mg/kg)	$4.64 \pm 1.79^{**}$	11.96 ± 0.54	5.83 ± 0.43	421.00 ± 162.20	$2.05 \pm 0.41^*$	39.54 ± 2.94	$0.67 \pm 0.17^*$

Values are presented as mean $\pm$ SEM ($n=5$) and analyzed by one-way ANOVA followed by Dunnet's *post hoc* test. $^{##}P < 0.01$ compared with the normal control group; $^*P < 0.05$, $^{**}P < 0.01$ compared to the colitis control group.

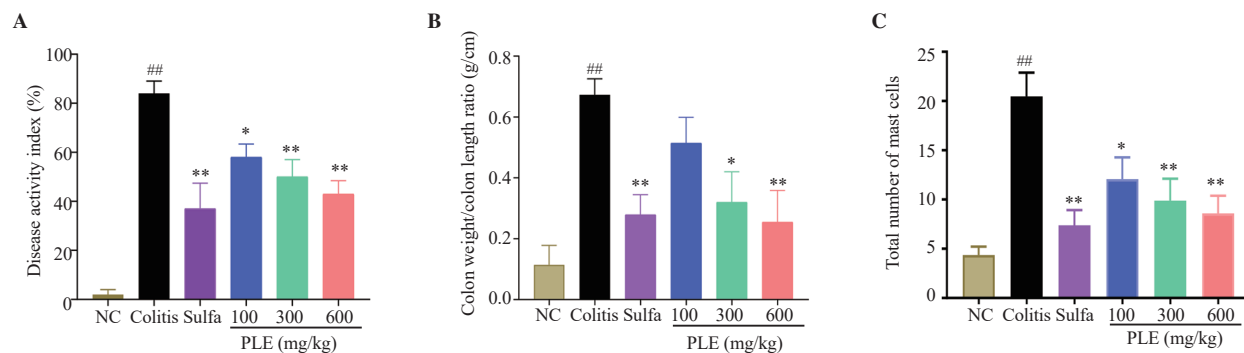


Figure 4. Effect of *Persicaria lanigera* extract on (A) disease activity index, (B) colon weight to colon length ratio as well as (C) mast cell proliferation in rats with acetic acid-induced ulcerative colitis. Values are presented as mean $\pm$ SEM ($n=5$) and analyzed by one-way ANOVA followed by Dunnet's *post hoc* test. $^{##}P < 0.01$ compared with the normal control group. $^*P < 0.05$, $^{**}P < 0.01$ compared with the colitis group.

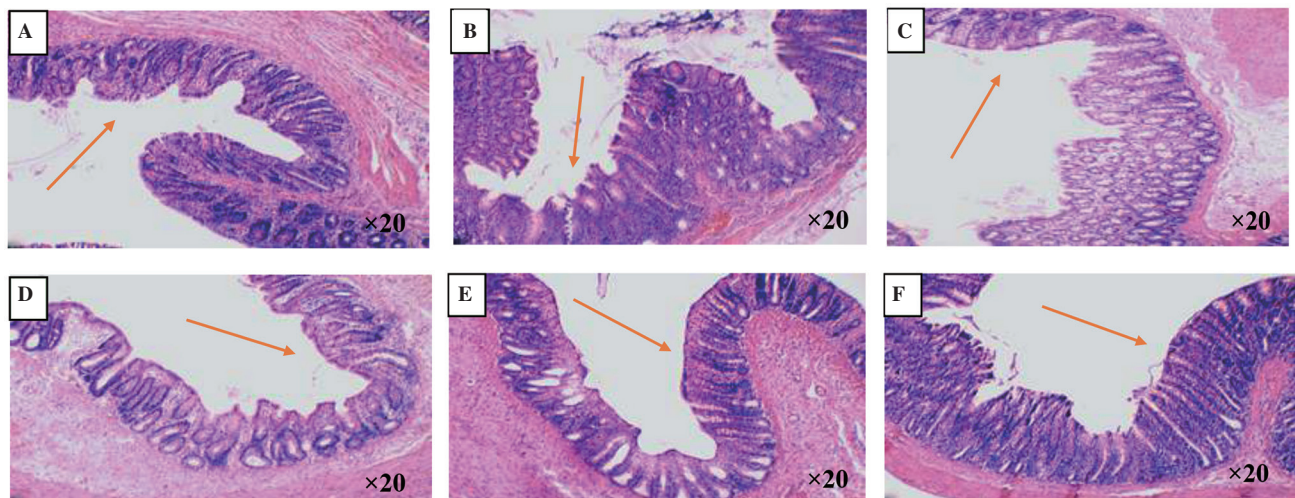


Figure 5. Effect of *Persicaria lanigera* on histopathological changes in rats with acetic acid-induced ulcerative colitis (magnification: $\times 20$). (A) Normal control, (B) colitis control, (C) the sulphasalazine-treated group, (D-F) *Persicaria lanigera* extract (100–600 mg/kg). The arrow shows the extent of colonic mucosal injury.

control group (Figure 4C). In contrast, *P. lanigera* extract decreased mast cell proliferation in a concentration-dependent manner ($P < 0.05$).

3.2.4. Effect of *P. lanigera* extract on hematological parameters

High levels of WBC and NEU in the acetic acid-induced colitis group were observed in relation to the normal control group (Table 3). Treatment with *P. lanigera* extract lowered WBC levels ($P < 0.05$) at all doses, LMP at 300 mg/kg ($P < 0.05$) and 600 mg/kg ($P < 0.05$), and NEU at 600 mg/kg ($P < 0.05$) compared with the negative control group (Table 3).

3.2.5. Effect of *P. lanigera* extract on acetic acid-induced histopathological alternations

The normal control group had intact mucosa, submucosa, and muscularis layers in the colon, with no signs of colonic inflammation (Figure 5A). The negative control group showed substantial colonic injuries, as evidenced by increased infiltrating inflammatory cells, mucosal ulceration, necrosis, edema, and epithelial disruption (Figure 5B). Sulfasalazine treatment ameliorated histological damage by considerably reducing cell necrosis, edema, inflammatory cell infiltration, and necrosis. Mucosal damage was inhibited, and the epithelium of the colon was protected compared to the negative control group (Figure 5C). Comparably, cell necrosis, edema, and inflammatory cell infiltration were significantly reduced in the *P. lanigera* extract-treated group. Mucosal ulceration was inhibited, and distortion of the colonic epithelium was prevented at 300 mg/kg and 600 mg/kg as compared with the negative control group (Figure 5D-F).

3.2.6. Effect of *P. lanigera* extract on antioxidant activities

The activities of catalase and SOD were drastically dropped in the negative control group ($P < 0.01$). Moreover, MDA level was significantly enhanced ($P < 0.01$), indicating increased oxidative stress. However, *P. lanigera* extract reversed acetic acid-induced alternations by substantially raising catalase and SOD activities and reducing MDA levels, especially at 600 mg/kg ($P < 0.01$) (Figure 2B).

4. Discussion

Inflammatory response to pathogens, chemicals, and mechanical or pernicious stimuli is chiefly targeted at averting tissue distress, attenuating internal disturbances, and ensuring homeostasis[14]. Nonetheless, this inflammatory protective reaction can lead to either acute or chronic inflammation but an unregulated process proceeds to numerous detrimental pathological conditions such as cancer, ulcers, cardiovascular disorders, asthma, and arthritis[2]. The

acute anti-inflammatory effects of *P. lanigera* have been reported[8], therefore, its effects on deregulated inflammatory responses such as cotton pellet-induced granuloma tissue formation and acetic acid-induced ulcerative colitis models in rats were investigated.

One of the recognized distinguishing characteristics of a chronic inflammatory response is the formation of granuloma tissue as a result of cotton pellet exposure[9,15]. To study the transudative, exudative, and proliferative phases of chronic inflammation, the cotton pellet-provoked granuloma tissue development concept is commonly used[16,17]. Increased vascular permeability causes fluid with low protein content to leak from blood vessels during the transudative stage, and an altered vascular permeability-related repair mechanism causes fluid with high protein content to infiltrate blood vessels during the exudative stage. The proliferative stage, which is the last stage, lasts for 3 to 6 d and is marked by the generation of granulomatous tissue as a result of the production of pro-inflammatory mediators[17,18].

The production of lysosomal enzymes, reactive oxygen species (ROS), and inflammatory mediators like IL-1, TNF- α , IL-6, eicosanoids, and chemokines are all responsible for the development of granulomatous tissue[18]. The proliferative stage is correlated with a rise in dry weight, while the transudative phase assesses the change in the wet weight of cotton pellets[19]. In the present study, *P. lanigera* extract exhibited a significant decrease in both dry and wet weights of granuloma tissue which confirmed its inhibitory effects on the transudative as well as proliferative phases of inflammatory response. This may suggest the antiproliferative action of *P. lanigera* extract on pro-inflammatory cytokines such as IL-1, TNF- α , and IL-6, the growth of fibroblasts, infiltration of neutrophils and monocytes, and the production of collagen and mucopolysaccharides, which are essential elements in the development of granuloma tissue[18]. *P. lanigera* extract elevated catalase and SOD activities, as well as reduced MDA levels, therefore inhibiting the production of ROS as a consequence of mediator release during the inflammatory response [20,21].

Most notably, the evaluation of hematological markers is essential to interpreting the blood-related effects of plant extracts[22]. Moreover, a blood indicator analysis provides crucial details regarding the body's reaction to infection, injury, stress, or deprivation by showing changes in metabolic activity and providing a clinical risk assessment of hematological abnormalities[23]. Increased WBC migration to the site of inflammation and higher WBC counts serve as inflammatory markers during an inflammatory response[24]. In addition, because they are crucial in inflammation, blood markers including NEU, LMP, and PLT are important for assessing the severity of diseases. Moreover, it has been demonstrated that NEU boost the production of cytokines like IL-1, IL-6, and TNF in addition to proteases and nitric oxide[25]. The results showed that WBC, NEU, and PLT levels were lower in the *P. lanigera* extract-treated group, which limited the inflammatory

activities of these blood cells. The extract also restored all other hematological parameters to normal[26] as compared to the negative control group.

The spleen enlarges as a result of its phagocytosis in the development of granuloma tissue caused by the cotton pellets[27]. In line with other studies, *P. lanigera* extract significantly decreased spleen weight when compared to the enlarged spleen of the control group[28]. Hence, *P. lanigera* extract inhibits the spleen's phagocytic activity during inflammatory reactions.

Inflammatory bowel disease (IBD) can be explored using the well-known model of ulcerative colitis caused by acetic acid[29], which may have certain pathophysiological traits with human IBD but differs etiologically. IBD patients frequently exhibit pathophysiological symptoms similar to those seen in rats after receiving an intrarectal injection of 3%-5% acetic acid, including mucosal ulceration, edema, weight loss, diarrhea, and bleeding[30]. Moreover, the condition is characterized by an elevated NEU infiltration into the damaged colon, mucosal and submucosal colon damage, the biosynthesis of pro-inflammatory mediators like cytokines and prostaglandins as well as the formation of ROS that cause oxidative damage[31].

Colonic damage is reported to cause bowel wall hardening, shrank colon length, a rise in colon weight-to-length proportion[32] and body weight loss. Due to protein loss, metabolic stress, malnutrition, malabsorption, or maldigestion, as well as excessive bodily fluid loss through diarrhea and colorectal bleeding brought on by intestinal inflammation, colitis patients may experience weight loss[29]. In this study, treatment with *P. lanigera* extract at 600 mg/kg increased body weight, however, there was a general loss of body weight observed and this is in line with a clinical study previously conducted on ulcerative colitis[29].

A macroscopic examination of the colon revealed that there had been a considerable colonic injury, which might be explained by increased inflammatory cell infiltration, necrosis, severe colon-wall edema, and goblet cell hyperplasia[33]. *P. lanigera* extract-treated rats showed improved colon weight, colon length shortening, stool consistency, and reduction of colonic damage. There were no mucus stains, visible blood, or loss of blood seen, and this may be related to the protection of the mucus layer and suppression of large loss of blood, which is a hallmark of anti-ulcerative compounds[34]. *P. lanigera* extract also significantly reduced the DAI score and preserved mucosal integrity.

It is known that anomalies in hematological variables are characteristic indications of ulcerative colitis. Hence, the evaluation of hematological parameters can be employed to assess the intensity of colitis[35]. In the present study, the acetic acid-induced colitis group showed a substantial rise in WBC and NEU concentrations. These variations in the hematological parameters contributed to the extensive mucosal ulcerations and therefore, treatment with *P. lanigera* extract significantly ameliorated these changes, which

agrees with the results of earlier studies[36].

Regarding histological examination, microscopic lesions in colonic tissues, as well as other histological complications, are seen after intrarectal injection of acetic acid in animals[37]. In this study, acetic acid-induced colonic damage was characterized by vital histological features such as increased inflammatory cellular infiltration, thickening of the smooth muscle, damaged mucosal layer, edema of the submucosal, necrosis, epithelial damage, and ulceration[38]. Treatment with *P. lanigera* extract ameliorated acetic acid-induced histological alternations and conferred protective effect on the mucosal layer of the colon. Moreover, the protective ability of this plant extract could be attributed to the significant suppression of cellular infiltration of inflammatory cells, ulcerations, necrosis, edema of the submucosal, distortions in the goblet cells, and enhanced regeneration of the crypt structures.

Mast cells are inflammatory cells that can be degranulated or appear intact. When activated, they degranulate and discharge several inflammatory mediators. Such cells, often highly expressed in inflamed colons, contribute to the disease status of ulcerative colitis[39]. In this study, intrarectal administration of acetic acid causes an enormous deposition of mast cells into the site of colonic injury. *P. lanigera* extract dramatically lowered the deposition of mast cells at the site of colonic injury consistent with the lower mast cell count observed in the study.

Oxidative stress is a major factor in the etiology of many diseases including diabetes, ulcer, cancer, and cardiovascular conditions[40]. It is known that MDA, a metabolic product of lipid peroxidation, increases due to oxidative stress[41], and a chain of antioxidant agents like SOD, catalase, and GSH act on ROS or free radicals after they have been produced. Thus, reducing ROS and free radicals is vital in chronic pathological conditions[42]. According to the study, *P. lanigera* extract inhibited oxidative stress by upregulating SOD and catalase activities while decreasing MDA content. The antioxidant activity of the plant might be attributed to its secondary metabolites reported in earlier studies which have shown plants contain derivatives of polyphenolic, saponins, and flavonoids with antioxidant activities[43]. However, further studies are needed to confirm the compounds responsible for its antioxidant and anti-inflammatory properties.

In conclusion, the leaf extract of *P. lanigera* possesses antiproliferative, antioxidant, and anti-colitis effects. This could add scientific evidence to support its folkloric use in traditional therapy for the management of inflammatory disorders.

Conflict of interest statement

The authors declare that they have no conflict of interest.

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Data availability statement

The data supporting the findings of this study are available from the corresponding authors upon request.

Authors' contributions

MAA, EO and EOA were involved in the conceptualization, design, definition of intellectual content, data acquisition, data analysis, manuscript preparation, interpretation, editing and review of the manuscript. MAA, EAA, RAA, and DA were involved in the experimental studies, literature search, collection of the plant materials, data analysis, statistical analysis, manuscript preparation, editing and review of the manuscript. MAA and AAK was involved in the execution of the ELISA work, editing and manuscript review. BA was involved in the histopathological analysis, editing and manuscript review.

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