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Protective Effect of *Paronychia argentea* L. on Acetic Acid Induced Ulcerative Colitis in Mice by Regulating Antioxidant Parameters and Inflammatory Markers

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ABSTRACT

The present study is designed to evaluate the antioxidant and anti-inflammatory effects of *Paronychia argentea* methanolic extract against acetic acid-induced ulcerative colitis in mice. The experimental colitis in mice was induced with 5% acetic acid (v/v, in saline) via rectal route. Animals were then divided into various treatment groups (n = 12). Four groups were treated with *P. argentea* methanolic extract (PAME) (50, 100, 200 and 500 mg/kg) for 5 consecutive days, and one group was treated with sulfasalazine (SASP, 500 mg/kg) as a standard drug. Disease activity index (DAI), colon weight and length, hematological parameters (C-reactive protein: CRP, erythrocyte sedimentation rate: ESR and full blood count: FBC), colonic contents of Malondialdehyde (MDA) and reduced glutathione (GSH), colonic superoxide dismutase (SOD) and catalase (CAT) activities were recorded. Results revealed that the intrarectal instillation of acetic acid caused increased DAI, colon weight, CRP, ESR and colonic MDA levels, and a decrease in the other parameters such as colon length, FBC, colonic GSH levels, SOD and CAT activities. Treatment with PAME showed significant effect in lowering DAI, colon weight, CRP, ESR and colonic MDA levels, and in enhancing the colon length, FBC, colonic GSH levels, SOD and CAT activities in a dose-dependent manner. These findings suggest that the extract obtained from *P. argentea* possess active substances, which exert marked protective effects in acute experimental colitis by regulating inflammatory markers and antioxidant parameters. *Paronychia argentea* may serve as a natural antioxidant and anti-inflammatory therapy for ulcerative colitis.

Key words: *Paronychia argentea* L.; ulcerative colitis; antioxidant activity; anti-inflammatory activity.

INTRODUCTON

Paronychia argentea L. Caryophyllaceae is one of the most used plants in folk medicine in Algeria, and is popularly known as Arabic tea (Kassaret lahdjer, Fettatet lahdjer or Bissat elmoulouk). *Paronychia argentea* was reported to contain the flavonoids isorhamnetin, quercetin, and luteolin [1]. The aerial parts of this plant are used in the Algerian traditional medicine as diuretic and for the treatment of renal diseases, especially as antiurolithiasis [2]. In addition, the plant was reported to have digestive [3], hypoglycemic [4], and antimicrobial activity [5]. Furthermore, Dafni *et al.* [6] reported the use of leaf decoction of this plant as a diuretic, in the treatment of kidney stones, diabetes, and heart ailments, and was also used as a blood purifier [7]. In Portugal, *Paronychia argentea* is used as analgesic, for stomach ulcer, anorexia, and flatulence [8].

On the other hand, ulcerative colitis (UC) is an inflammatory bowel disease (IBD) that primarily affects the colon and rectum. It features a recurrent, with chronic relapsing conditions in the intestine [9]. The clinical manifestations include diarrhea, blood in the stool, abdominal pain, and weight loss [10]. The prescribed drugs of choice for colitis are mesalazine and sulfasalazine [11]. However, due to the safety of sulfasalazine decays in patients with known tolerance, these drugs can cause unwanted effects on male infertility [12]. Additionally, the adverse effects of sulfasalazine include vomiting, hypospermia, hepatitis, pneumonitis, hemolytic anemia, chronic nephrosis and encephalitis [13], and in rare cases, sulfasalazine can aggravate colitis, resulting in diarrhea, abdominal cramps and discomfort [14]. Medicinal plant based therapy can possess a protective effect, with less or no adverse effects, against colitis by causing an elevation in antioxidants and the activities of associated enzymes [15]. Therefore, the use of traditional medicinal plants products may provide therapeutic alternatives or supplementary treatment of patients, and the structures of these products can serve to provide leads for the synthesis of new anti-inflammatory agents [16].

Oxidative stress could be a major contributing factor to tissue injury and fibrosis that characterize the inflammatory bowel disease. An increased level of superoxide and nitric oxide, NO, during ulcerative colitis, increases peroxynitrite formation that mediates oxidation of lipids, proteins, and DNA [17]. Chronic inflammation, on the other hand, increases oxidative stress, regeneration, repair, and dysplasia in the colon that transform into an invasive colorectal cancer (CRC) [18]. To scavenge reactive oxygen species (ROS), gastric cells have several enzymatic and non-enzymatic antioxidants including superoxide dismutase (SOD), reduced glutathione (GSH) and catalase (CAT), however, excessive generation of ROS enhances lipid peroxides (LPO) and could deplete these antioxidant enzymes [19]. *In vitro* and *in vivo* studies have concluded that flavonoids are appropriate candidates of new anti-inflammatory drugs for scavenging the reactive oxygen species, reducing the damage to tissues, and affecting the signal transduction pathways [20]. In addition to their direct radical scavenging effects, polyphenols are thought to be able to enhance endogenous antioxidative capacity [21]. As for the plant, *Paronychia argentea* L., Zama and coworkers [2], studied the protective effect of a butanol extract of *Paronychia argentea* L. against toxicity caused by the organophosphorus pesticide, chloropyriphos ethyl in pregnant female rats using different parameters such as plasma and tissue malondialdehyde, blood reduced glutathione and erythrocyte superoxide dismutase. Accordingly, in the present work we employed *in vivo* assays to evaluate the antioxidant activity and the anti-inflammatory effect of *Paronychia argentea* methanolic extract against acetic acid-induced ulcerative colitis in mice, which to the best of our knowledge has not been explored.

MATERIALS AND METHODS

Plant Material

Aerial parts of *Paronychia argentea* were collected, at the flowering stage (April - May 2011), from Ouled Rahmoune (mountainous region, Constantine, Algeria) and air-dried for 10 days at room temperature. Prof. Oudjehih Bachir, a botanist at the University of El Hadj Lakhdar, Batna, Algeria, identified and authenticated the plant.

Animals

Male *Swiss albino* mice (20-30 g) were obtained from the Laboratory of Animal Physiology, Constantine 1 University, Algeria. These animals were housed at constant temperature of 25 ± 2 °C with a 12 h light/dark cycle. They were given free access to tap water and standard diet. All chemicals were purchased from Sigma (Germany), Pfizer Health AB (Sweden), Prolabo, Aldrich and Fluka. Mice were randomly divided into seven groups of 12 animals each. Group 1: normal control (NC) received 0.9% saline (2 mL/kg, i.p.). Group 2: colitis control (CC) received 0.9% saline (2 mL/kg, i.p.) following induction of colitis. Groups 3, 4, 5 and 6 were given *P. argentea* methanol extract (PAME) 50, 100, 200, 500 mg/kg, respectively 2 h following induction of colitis. Group 7: received sulfasalazine (SASP), (500 mg/kg). Drugs were suspended in saline and administered orally once daily.

Preparation of *P. argentea* methanolic extract

Approximately 500 g of powdered plant materials was soaked, at room temperature, in 5 L of methanol for 48 h with occasional stirring [22]. Then the mixture was filtered and the filtrate was concentrated under reduced pressure with a rotary evaporator. The extract was further concentrated by allowing it to stand overnight in an oven at 30 °C, and was then stored until use.

Total polyphenol content determination

Total polyphenol content of *Paronychia argentea* methanolic extract (PAME) was determined with the aid of Folin-Ciocalteu reagent using gallic acid as a standard according to Li *et al.* [23]. In brief, 0.1 mL of PAME was mixed with 2.5 mL of distilled water and 0.5 mL of the Folin-Ciocalteu stock reagent. After 5 min, 1.0 mL of 20% aqueous Na_2CO_3 solution was added to the mixture. The mixture was then incubated at room temperature for 1h and its

absorbance was measured at 760 nm. The amount of total polyphenols in the extract was determined from standard curves of gallic acid and results are expressed in milligrams of gallic acid equivalents per gram of dried PAME.

Total flavonoid content determination

Total flavonoid content in PAME was determined according to the method of Bahorun *et al.* [24]. This method is based on the formation of a flavonoid-aluminum complex when the flavonoid-containing extract is mixed with an aqueous solution of aluminum chloride. Total flavonoid content was determined by measuring the absorbance of the extract-aluminum chloride mixture at 430 nm; quercetin and rutin were used as standards. Briefly, 1 mL of each sample was mixed with 1 mL of aluminium chloride (AlCl₃) solution (2%) in methanol. After an incubation period of 10 min, absorbance of mixture was measured at 430 nm versus a prepared methanol blank. Results are expressed as milligrams of quercetin and rutine equivalents per gram of dried PAE.

Antioxidant and anti-inflammatory activities (acetic acid-induced colitis assay)

Induction of experimental colitis

Acute colitis was induced by acetic acid according to Neurath *et al.* [25] and Rachmilewitz *et al.* [26] with slight modifications. Briefly, 24 h starved mice were lightly anesthetized with ether, and then a metal catheter was carefully inserted 4 cm into the colon *via* the anus. One millilitre of acetic acid (5% v/v in 0.9% saline) was slowly instilled into the lumen of the colon. Animals were then maintained in a head down position for 30 s to limit expulsion of solution. In normal control experiments, mice received 0.9% saline alone using the same method.

On the 6th day, animals were anesthetized with ether and blood was collected by retro orbital puncture for measuring inflammatory markers: erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and full blood count (FBC). Animals were then sacrificed and colonic segments were excised, freed of adherent adipose tissue, washed with cool saline, and were used for clinical scoring. Colonic samples were homogenized in KCl (1.15 %) with the aid of an ultrasonic homogenizer and homogenates were centrifuged at 4000 rpm for 10 min at 4 °C. The supernatants were frozen at -20 °C until use for the determination of total protein and for antioxidant parameters (MDA and GSH levels, CAT and SOD activities).

Clinical activity score

Body weight, stool consistency, and gross bleeding were recorded daily. Each score was determined according to the method of Cooper *et al.* [27] as follows: change in body weight loss (0: none, 1: 1-5%, 2: 5-10%, 3: 10-20%, 4: >20%); stool blood (0: negative, 2: hemocult positive, 4: gross bleeding); and stool consistency (0: normal, 1 and 2: loose stool, 3 and 4: diarrhea). Disease activity index (DAI) was quantified with a clinical score which records weight loss, stool consistency, and bleeding of the colon. Body weight loss was calculated as the percent difference between the original body weight (day 0) and the body weight on any particular day. At the end of the experiment, mice were sacrificed and the colons were separated from the proximal rectum, close to its passage under the pelvisternum. The colon length was measured between the ileo-cecal junction and the proximal rectum and its weight was measured.

Inflammatory markers measurements

Erythrocyte sedimentation rate (ESR) values were measured using the Westergren method described by Westergren [28] and Fahraeus [29]. Briefly, 0.8 mL (4 volumes) of the blood samples collected in tubes containing EDTA (1 mg/mL of blood) for each group of mice were mixed with 0.2 mL (1 volume) of a sodium citrate solution (3.8%). Westergren tubes (Westergren graduated pipettes) were filled with citrated blood, and placed vertically into the Westergren supports. Erythrocyte sedimentation in mm was recorded at one hour interval for 2 hours and compared with those of other groups.

C-reactive protein (CRP) concentrations were determined according to the manufacturer's recommendations (Spinreact) using the agglutination technique (CRP-Latex). Briefly, 50 µL of serum samples of each group of mice were deposited on the circles of test plates, one drop (50 µL) of CRP-Latex reagent was subsequently deposited beside each previous drop. Each two drops (serum / reagent) were mixed by rotation with the aid of a wand, trying to expand the mixture over the entire inside area of the circle. After stirring for 2 min, the presence or absence of agglutination was examined by the naked eye. The presence of agglutination indicates a CRP concentration ≥ 6 mg/L, whereas the absence of agglutination shows a CRP concentration < 6 mg/L.

Analyses of hematological parameters (FBC) of blood samples, collected in tubes containing EDTA (1 mg/mL of blood) for each group of mice, were carried out using a self-hematology analyzer (Beckman coulter), at the central laboratory of El Khroub hospital, Constantine, Algeria.

Antioxidant studies**Malondialdehyde and glutathione measurements**

Lipid peroxidation was evaluated by measuring malondialdehyde (MDA) levels using the procedure outlined by Okhawa *et al.* [30]. In this method, an aliquot (0.5 mL) of the colon homogenate was added to 0.5 mL of 20% TCA and 1 mL of 0.67% TBA. The mixture was boiled for 15 min at 100 °C, cooled immediately in ice, and added to 4 mL of *n*-butanol. It was then centrifuged at 3000 rpm for 15 min, and the absorbance of the supernatant was measured with a spectrophotometer at 530 nm and compared with a curve made by MDA standards (serial dilutions of the stock 10 mM 1,1,3,3-tetraethoxypropane solution). The amount of MDA was expressed as nmol/g of colonic tissues.

On the other hand, colon glutathione (GSH) level was determined according to Ellman [31]. GSH in colonic tissue homogenate was measured by its reaction with the Ellman's reagent, 5,5'-dithio-bis-(2-nitrobenzoic acid), (DTNB). A volume of 0.5 mL of 10% trichloroacetic acid (TCA) was added to 0.5 mL of colonic tissue homogenate. After 15 min, each sample was centrifuged (2000 rpm, 5 min) and the supernatant was analyzed for GSH. A volume of supernatant (0.2 mL) was combined with 1.7 mL of 0.1 M potassium phosphate buffer (pH 8), and then 0.1 mL of DTNB was added to the tubes and the intensity of the resulting yellow color was measured after 5 min at 412 nm. GSH concentrations were calculated using the standard curve of GSH, and results are expressed as $\mu\text{mol/g}$ of colonic tissues.

Determination of superoxide dismutase activity

The activity of superoxide dismutase (SOD) was determined using the method of Misra and Fridovich [32] with slight modifications. According to this method, 0.2 mL of colonic tissue homogenate was added to 2.5 mL of 0.05 M carbonate buffer (pH 10.2). The reaction was initiated by the addition of 0.3 mL of freshly prepared 0.3 mM adrenaline. An increase in absorbance was recorded at 480 nm every 30 s for 150 s. One unit of SOD activity induced approximately 50% inhibition of the auto-oxidation of adrenaline to adrenochrome in 1 min. Results are expressed as UI/mg protein. **Determination of catalase (CAT) activity**

Activity assay was based on the ability of CAT to induce the disappearance of H_2O_2 according to the method described by Clairborne [33]. The reaction mixture contained 50 μL of homogenate and 2.95 mL of H_2O_2 (0.019 M in potassium phosphate buffer 0.1 M, pH 7.4), and the absorbance was read at 240 nm at 1 min interval for 2 min using UV-visible spectrophotometer. One unit of CAT activity was calculated by using the following formula:

$$k = (2.303/\Delta t) \times \log (A1/A2)$$

where: k is the first order reaction rate constant, Δt : time interval in min, $A1$: absorbance in time 1, $A2$: absorbance after 1 min. CAT activity was then calculated as k/m , where m is the mass of protein in the used volume of colonic sample in mg. CAT activity was expressed as UI/mg protein.

Protein determination

Protein concentration in the colon supernatants was measured using the biuret reagent [34] using bovine serum albumin as a standard.

Statistical analysis

All determinations in this investigation were conducted in triplicate and results are expressed as mean \pm standard error of the mean (SEM). In this study, statistical analysis was performed using Student's *t*-test for significance, and analysis of variance (ANOVA) followed by Dunnett's test were done for the multiple effects comparison; p values less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION**Extraction and polyphenols and flavonoids contents**

Extraction was carried out using methanol, results showed that the plant extract has a yield of about 10.971 ± 0.637 %. In addition, results also showed that this extract contained significant polyphenol (217.463 ± 0.87 mgGA-Eq/gE) and flavonoid (13.349 ± 0.562 mgQ-Eq/gE, 26.765 ± 0.837 mgR-Eq/gE) contents.

Antioxidant and anti-inflammatory activities (acetic acid-induced colitis assay)**General observation and clinical activity score**

Colonic instillation of acetic acid triggered intense inflammatory responses in the large bowel, characterized by extensive hemorrhage, occasional ulceration, bowel wall thickening, and high macroscopic damage. Administration of saline did not protect against colonic injury [35]. In the present study, acetic acid-treated mice groups developed

rapidly severe colitis with typical signs including: bloody diarrhea, reduced mobility and food intake, poor coat quality and dramatic body weight loss. Gross blood adhesion to the anus was observed in some mice in addition to a high mortality rate. Treatment with extracts increased survival rate of mice in a dose-dependent manner as displayed in Figure 1; no mortality was observed in the intact groups.

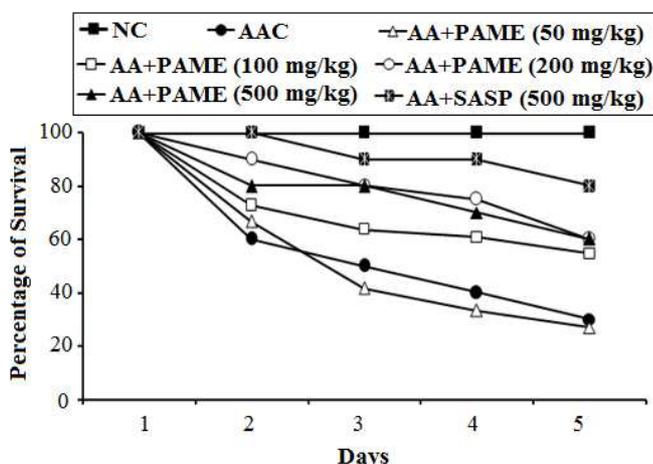


Figure 1. Effects of different doses of *P. argentea* methanolic extract (50, 100, 200 and 500 mg/kg) and sulfasalazine (500 mg/kg) on survival rate of mice with acetic acid-induced colitis, compared with normal control (NC) and acetic acid control (AAC) groups

As shown in Table 1, the administration of PAME and SASP prevented reduction of body weight. Blood appeared in the stool of all experimental groups treated with acetic acid just few minutes after induction of colitis (Table 2). Changes in the consistency of the stool were observed immediately after administration of acetic acid in all groups (Table 3); none of the described changes were observed in the intact group.

Table 1. Effects of different doses of *P. argentea* methanolic extract and sulfasalazine on body weight loss score in mice with acetic acid-induced colitis

Body weight loss score	Day 1	Day 2	Day 3	Day 4	Day 5
NC	0	0	0	0	0
AAC	0	2.4 ± 0.032 [#]	3 ± 0.041 ^{###}	3.5 ± 0.094 ^{####}	3.96 ± 0.32 ^{####}
PAME (50 mg/kg)	0	2.33 ± 0.037 ^{ns}	2.66 ± 0.075 ^{ns}	3.33 ± 0.035 ^{ns}	3.93 ± 0.29 ^{ns}
PAME (100 mg/kg)	0	1.16 ± 0.049 ^{ns}	1.833 ± 0.058 ^{ns}	2.0 ± 0.075 [*]	2.0 ± 0.055 ^{**}
PAME (200 mg/kg)	0	0.83 ± 0.014 ^{ns}	1.33 ± 0.036 [*]	1.83 ± 0.085 ^{**}	1.66 ± 0.065 ^{***}
PAME (500 mg/kg)	0	0.75 ± 0.018 ^{ns}	1.33 ± 0.089 [*]	1.91 ± 0.09 ^{**}	1.6 ± 0.039 ^{***}
SASP (500 mg/kg)	0	0.66 ± 0.028 [*]	1.16 ± 0.016 ^{**}	1.5 ± 0.014 ^{**}	1.33 ± 0.004 ^{***}

Data are expressed as means ± SEM (n ≥ 3). [#]p < 0.05, ^{###}p < 0.01 and ^{####}p < 0.001 acetic acid control vs. normal control; ^{ns}p > 0.05, ^{*}p < 0.05, ^{**}p < 0.01 and ^{***}p < 0.001 vs. acetic acid control.

Table 2. Effects of different doses of *P. argentea* methanolic extract and sulfasalazine on stool blood score in mice with acetic acid-induced colitis

Stool blood score	Day 1	Day 2	Day 3	Day 4	Day 5
NC	0	0	0	0	0
AAC	4 ± 0 ^{###}	4 ± 0.032 ^{###}	3.8 ± 0.04 ^{###}	3.75 ± 0.094 ^{###}	3.66 ± 0.32 ^{###}
PAME (50 mg/kg)	4 ± 0 ^{ns}	3.66 ± 0.037 ^{ns}	4 ± 0.037 ^{ns}	4 ± 0.037 ^{ns}	3.75 ± 0.29 ^{ns}
PAME (100 mg/kg)	4 ± 0 ^{ns}	3.16 ± 0.05 ^{ns}	3 ± 0.049 ^{ns}	2.25 ± 0.075 ^{**}	0.5 ± 0.045 ^{****}
PAME (200 mg/kg)	4 ± 0 ^{ns}	2.8 ± 0.014 ^{ns}	2.2 ± 0.036 ^{**}	1.6 ± 0.085 ^{***}	0.2 ± 0.065 ^{****}
PAME (500 mg/kg)	4 ± 0 ^{ns}	3.16 ± 0.02 ^{ns}	2.3 ± 0.048 [*]	1.7 ± 0.093 ^{***}	0.25 ± 0.039 ^{****}
SASP (500 mg/kg)	4 ± 0 ^{ns}	2.58 ± 0.028 [*]	1.7 ± 0.016 ^{***}	0.6 ± 0.014 ^{****}	0 ^{****}

Data are expressed as means ± SEM (n ≥ 3). ^{###}p < 0.001 acetic acid control vs. normal control; ^{ns}p > 0.05, ^p< 0.05, ^{*}p < 0.01, ^{**}p < 0.001 and ^{****}p < 0.0001 vs. acetic acid control.

Table 3. Effects of different doses of *P. argentea* methanolic extract and sulfasalazine on stool consistency score in mice with acetic acid-induced colitis

Stool consistency score	Day 1	Day 2	Day 3	Day 4	Day 5
NC	0	0	0	0	0
AAC	4 ± 0 ^{###}	3.8 ± 0.44 ^{###}	3.71 ± 0.4 ^{###}	3.6 ± 0.82 ^{###}	3.2 ± 0.32 ^{###}
PAME (50 mg/kg)	4 ± 0 ^{ns}	3.75 ± 0.64 ^{ns}	3.75 ± 0.21 ^{ns}	3.75 ± 0.65 ^{ns}	3.5 ± 0.19 ^{ns}
PAME (100 mg/kg)	4 ± 0 ^{ns}	3.54 ± 0.57 ^{ns}	2.9 ± 0.56 ^{ns}	2.16 ± 0.39 ^{**}	1.16 ± 0.34 ^{***}
PAME (200 mg/kg)	4 ± 0 ^{ns}	3.2 ± 0.48 ^{ns}	2.28 ± 0.69 ^{**}	1.5 ± 0.34 ^{***}	0 ^{****}
PAME (500 mg/kg)	4 ± 0 ^{ns}	3.0 ± 0.8 ^{ns}	2.42 ± 0.98 ^{**}	1.2 ± 0.4 ^{***}	0 ^{****}
SASP (500 mg/kg)	4 ± 0 ^{ns}	2.5 ± 0.76 ^{**}	1.62 ± 0.62 ^{***}	0.71 ± 0.55 ^{****}	0 ^{****}

Data are expressed as means ± SEM (n ≥ 3) ^{###}p < 0.001 acetic acid control vs. normal control; ^{ns}p > 0.05, ^{*}p < 0.05, ^{**}p < 0.01, ^{***}p < 0.001 and ^{****}p < 0.0001 vs. acetic acid control.

Additionally, PAME significantly reduced the disease activity index (DAI), a clinical parameter reflecting the severity of weight loss, rectal bleeding, and stool consistency (Fig. 2). Acetic acid caused severe macroscopic edematous inflammation in the colon, and caused colonic shortening (Fig. 3a) and decrease of its wet weight (Fig. 3b) in the colitis control group. However, such changes were improved by treatment with 100, 200, and 500 mg/kg of PAME; treatment with 50 mg/kg had no effect, and the SASP (500 mg/kg) showed better results in these parameters, indicating its potent activity at the tested dose.

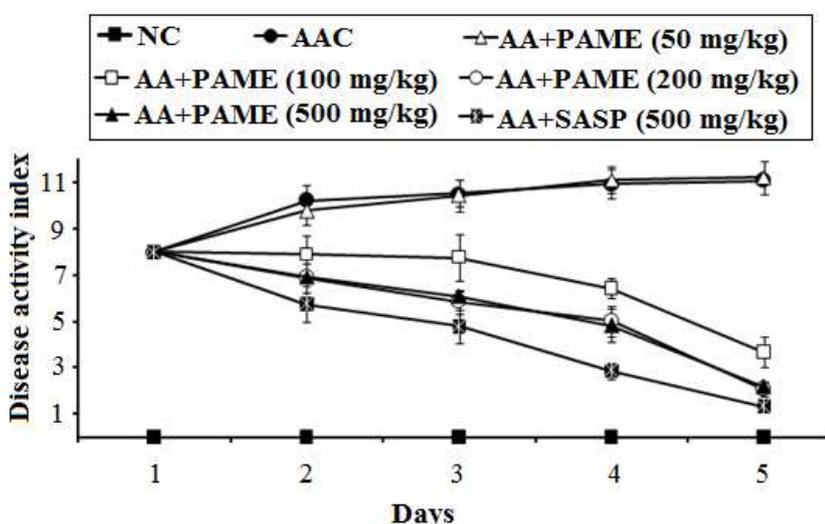


Figure 2. Effect of different doses of *P. argentea* methanol extract (50, 100, 200 and 500 mg/kg) and sulfasalazine (500 mg/kg) on disease activity index (DAI) of mice with acetic acid-induced colitis, compared with normal control (NC) and acetic acid control (AAC) groups. Data are expressed as means ± SEM (n ≥ 3). ^{###}p < 0.001 acetic acid control vs. normal control; ^{ns}p > 0.05 and ^{*}p < 0.001 vs. acetic acid control**

Similarly, considerable increase in colon weight, weight/length ratio, diarrhea, rectal bleeding, macroscopic score, histological alterations, shearing of epithelial cells, and decrease in body weight were indicators for the severity of inflammation in colitis [36]. In our study, induction of acetic acid alone causes significant elevation in these parameters. Treatment with *P. argentea* methanolic extract leads to a dose-dependent decrease in the above mentioned parameters which indicate its anti-inflammatory and antiulcer activities.

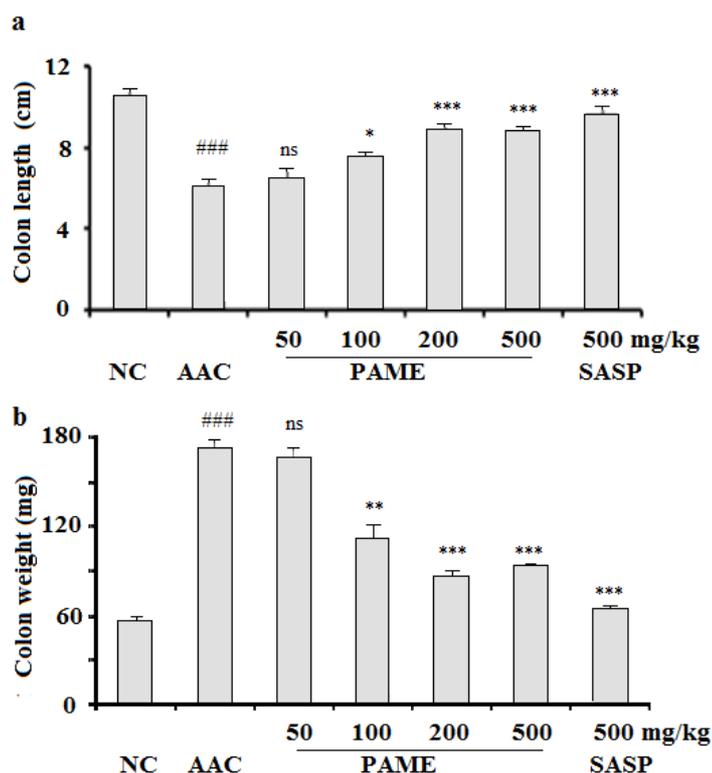


Figure 3. Effects of different doses of *P. argentea* methanolic extract (PAME) and sulfasalazine (SASP) on colon length (a) and weight (b) of mice with acetic acid-induced colitis, compared with normal control (NC) and acetic acid control (AAC) groups. Data are expressed as means \pm SEM ($n \geq 3$). ### $p < 0.001$ acetic acid control vs. normal control; ns $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ vs. acetic acid control

Measurement of inflammatory markers

The erythrocyte sedimentation rate (ESR) count has been used as a marker for inflammatory disease [37]. In order to find out the effect of PAME against inflammation, ESR count for 1 and 2 hours was done. Results presented in Figure 4 show that doses of 100, 200 and 500 mg/kg of PAME have the ability to reduce ($p < 0.05$) elevated levels of ESR after 1 and 2 hours. However, the colitic mice treated with 50 mg/kg of PAME had the same ESR level as the colitic mice control.

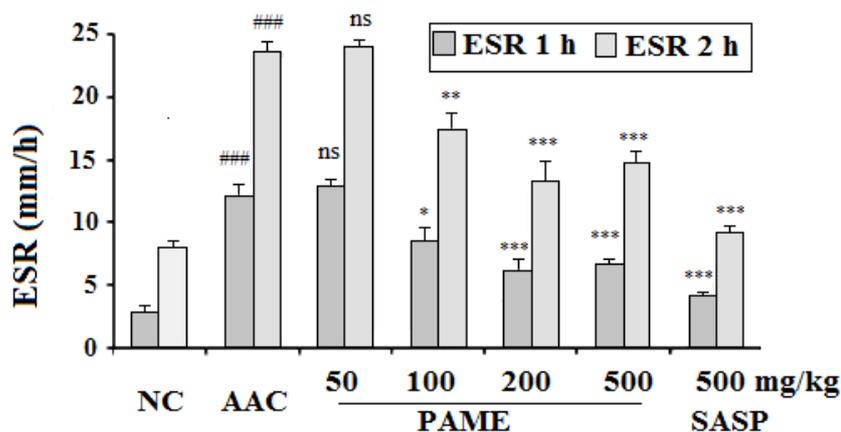


Figure 4. Effects of different doses of *P. argentea* methanolic extract and sulfasalazine on the erythrocyte sedimentation rate (ESR) 1st and 2nd hour of mice with acetic acid-induced colitis, compared with normal control (NC) and acetic acid control (AAC) groups. Data are expressed as means \pm SEM ($n \geq 3$). ### $p < 0.001$ acetic acid control vs. normal control; ns $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ vs. acetic acid control

Measurement of serum C-reactive protein levels is used to confirm the effect on inflammation [38], according to that, our results show that CRP decreased from 25.01 ± 2.041 mg/L in control colitic mice to 8.5 ± 0.95 mg/L in colitic mice treated with PAME (200 mg/kg); this effect was dose-dependent (Fig. 5). However, a dose of 50 mg/kg

of the plant extract did not show any significant effect (Fig. 5). The decreased severity of colitis in SASP (500 mg/kg) treated mice compared to colitis control group was further demonstrated by a 5 fold decrease in serum CRP level as shown in Figure 5.

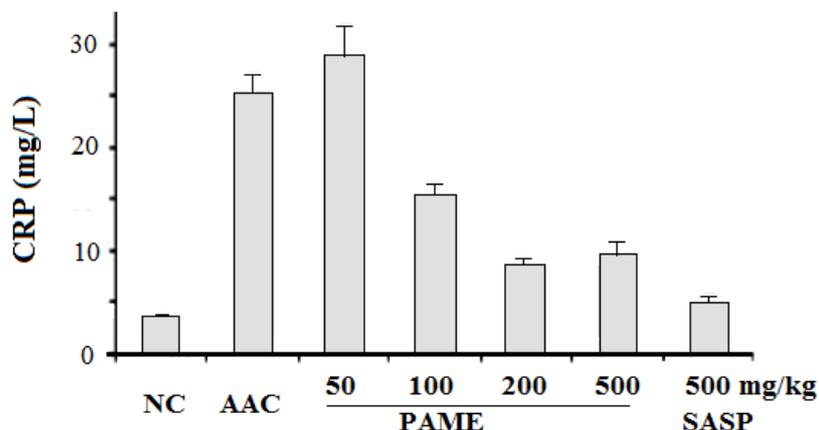


Figure 5. Effects of different doses of *P. argentea* methanolic extract and sulfasalazine on C-reactive protein (CRP) levels of mice with acetic acid-induced colitis, compared with normal control (NC) and acetic acid control (AAC) groups. Data are expressed as means \pm SEM ($n \geq 3$)

Similarly, clinical manifestations of inflammatory bowel disease includes exacerbated hematological imbalance leading to an unexplained diarrhea [39]. A full blood count (FBC) was performed, and results demonstrated that the acetic acid control mice showed significant decrease in white blood cells (WBC), red blood cells (RBC), hemoglobin (Hb), hematocrit (HCT) and platelets (PLA) as compared to normal mice as listed in Table 4. Treatment with 100, 200, and 500 mg/kg of PAME and 500 mg/kg SASP led to a significant increase in these hematological parameters as compared to colitic control mice. These results are in agreement with those of Gupta *et al.* [40].

Table 4. Effects of different doses of *P. argentea* methanol extract (PAME) and sulfasalazine (SASP) on full blood count (FBC) in mice with acetic acid-induced colitis (AAC)

Full blood count (FBC)	WBC ($\times 10^3/\mu\text{L}$)	RBC ($\times 10^6/\mu\text{L}$)	Hb (g/dL)	HCT (%)	PLA ($\times 10^5/\mu\text{L}$)
NC	11.03 \pm 0.54	10.01 \pm 0.65	15.83 \pm 0.44	43.57 \pm 0.33	14.6 \pm 0.45
AAC	4.67 \pm 0.089 ^{###}	4.84 \pm 0.30 ^{###}	9.86 \pm 0.24 ^{###}	30.07 \pm 0.63 ^{###}	7.54 \pm 0.31 ^{###}
PAME (50 mg/kg)	4.92 \pm 0.075 ^{ns}	5 \pm 1 ^{ns}	9.93 \pm 0.37 ^{ns}	32.95 \pm 1.45 ^{ns}	7.8 \pm 0.2 ^{ns}
PAME (100 mg/kg)	7.43 \pm 0.56 ^{**}	7.65 \pm 0.28 ^{**}	12.4 \pm 0.30 ^{**}	36.47 \pm 0.86 ^{**}	10.15 \pm 0.37 ^{**}
PAME (200 mg/kg)	9.16 \pm 0.44 ^{***}	8.75 \pm 0.35 ^{***}	13.97 \pm 0.14 ^{***}	39.97 \pm 0.60 ^{***}	12.07 \pm 0.073 ^{***}
PAME (500 mg/kg)	8.91 \pm 0.31 ^{***}	8.64 \pm 0.96 ^{***}	13.83 \pm 0.16 ^{***}	38.83 \pm 0.72 ^{***}	11.86 \pm 0.11 ^{***}
SASP (500 mg/kg)	10.3 \pm 0.51 ^{***}	9.14 \pm 0.77 ^{***}	14.67 \pm 0.28 ^{***}	42.2 \pm 0.8 ^{***}	13.53 \pm 0.84 ^{***}

Data are expressed as means \pm SEM ($n \geq 3$). ^{###} $p < 0.001$ acetic acid control vs. normal control, ^{ns} $p > 0.05$, ^{*} $p < 0.05$, ^{**} $p < 0.01$, and ^{***} $p < 0.001$ vs. acetic acid control. WBC: white blood cells, RBC: red blood cells, Hb: hemoglobin HCT: hematocrit, PLA: platelets

The ESR and the CRP levels are the most widely used tests to monitor and detect inflammatory disorders. They are both sensitive markers of inflammation and correlate with the severity of inflammation, but are not specific to any particular illness [41]. In the present investigation, treatment with PAME reduced the colon inflammation in a dose-dependently by decreasing the elevated levels of ESR and CRP, and elevating the reduced levels of FBC.

Antioxidant studies

MDA and GSH measurements

Free radicals and reactive oxygen species (ROS) were reported in colorectal specimens of ulcerative colitis [42]. Lipid peroxidation, an indicator of mucosal injury induced by reactive oxygen species, was measured as thiobarbituric acid reactive substance (TBARS). On the other hand, malondialdehyde (MDA), the product of lipid peroxidation, was estimated by reaction with thiobarbituric acid [43]. In this study, the change in MDA content between control group and PAME-treated animals is displayed in Fig. 6. No changes were observed in MDA content between colitis control and 50 mg/kg PAME-treated groups. In these two groups, animals exhibited increased levels of MDA in colon tissue. In the groups that received 100, 200, and 500 mg/kg of PAME and 500 mg/kg of SASP, MDA levels were very significantly reduced. The inhibitory activity of ulcerative colitis by *P. argentea* treatment may be related to the anti-lipid peroxidation and free radical scavenging ability.

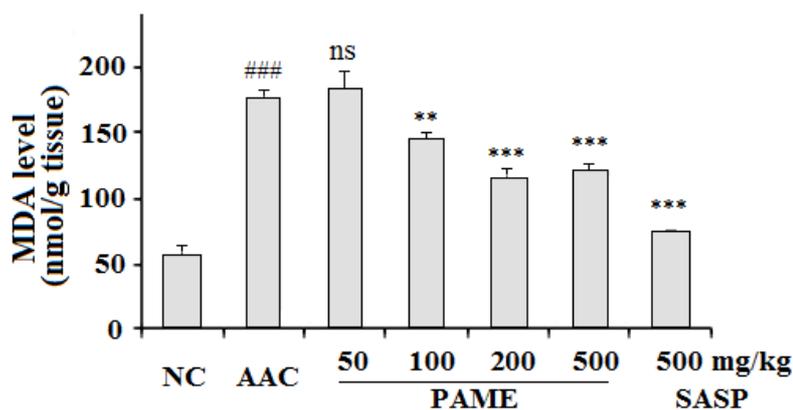


Figure 6. Anti-lipid peroxidation effects of different doses of *P. argentea* methanol extract and sulfasalazine by measuring colonic malondialdehyde (MDA) levels in mice with acetic acid-induced colitis, compared with normal control (NC) and acetic acid control (AAC) groups. Data are expressed as means \pm SEM ($n \geq 3$). ### $p < 0.001$ acetic acid control vs. normal control; ns $p > 0.05$, ** $p < 0.01$, and *** $p < 0.001$ vs. acetic acid control

Colonic inflammation, both in human IBD and in experimental colitis, is associated with increased release of reactive oxygen metabolites and nitric oxide levels [43]. In 2013, Karbinger and coworkers [44] have shown that changes in the oxidative state are manifested as an increase in the lipid peroxidation capacity and a decrease in the antioxidant defense capacity of the glutathione system. In our study, reduced glutathione (GSH) levels in the colonic tissue were determined. In agreement with the above reports, GSH levels were significantly reduced by the induction of colitis. However, administration of PAME significantly increased GSH levels when compared with UC control as given in Fig. 7. These results showed a significant protective effect which demonstrates the role of *P. argentea* and its use in IBD. In addition, this may also suggest the anti-inflammatory effect of *P. argentea*, which could be due to its ability to release glutathione and the antioxidant dipeptide γ -Glu-Cys [45].

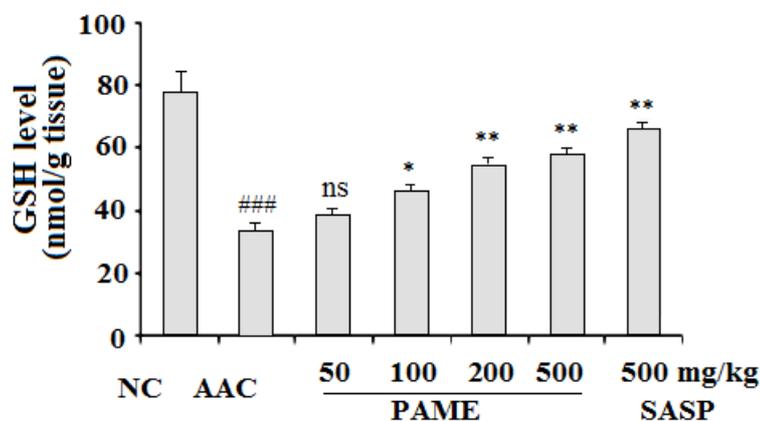


Figure 7. Effects of different doses of *P. argentea* methanolic extract and sulfasalazine on colonic reduced glutathione (GSH) levels of mice with acetic acid-induced colitis, compared with normal control (NC) and acetic acid control (AAC) groups. Data are expressed as mean \pm SEM ($n \geq 3$). ### $p < 0.01$ acetic acid control vs. normal control; ns $p > 0.05$, * $p < 0.05$ and ** $p < 0.01$ vs. acetic acid control

Determination of SOD activity

Superoxide dismutase (SOD) catalyzes dismutation of superoxide anion to hydrogen peroxide, which is subsequently converted to water by catalase. To regulate ROS levels, the intestinal mucosa possesses complex antioxidant systems where SOD is the primary antioxidant enzyme that converts superoxide anion to H_2O_2 [17]. The effect of different doses of *P. argentea* methanolic extract on SOD activity in the colonic tissue during ulcerative colitis (UC) is shown in Fig. 8. Our results are in agreement with those obtained by other workers [46]. SOD activity was significantly ($p < 0.001$) reduced in the colons of acetic acid (AA) treated animals compared with normal control mice (2.22 ± 0.097 UI/mg of protein vs. 5.096 ± 0.15 UI/mg of protein). No change in colonic tissue SOD activity level in the 50 mg/kg of PAME-treated group was observed when compared with UC control group, whereas groups treated with 100, 200, and 500 mg/kg of PAME and 500 mg/kg of SASP showed significant ($p < 0.01$ and $p < 0.001$) increase in SOD activities in colons compared with AA treated animals. This increased level could prevent the adhesion of circulating leucocytes to intestinal endothelium by blocking the expression of adhesion, such as vascular cell and intracellular adhesion molecules [47].

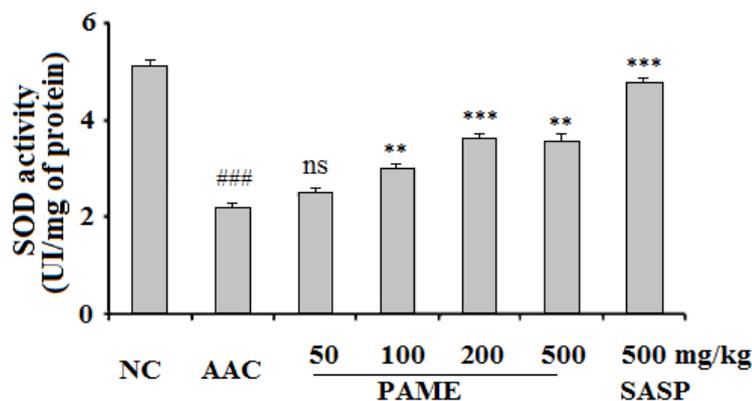


Figure 8. Effect of different doses of *P. argentea* methanolic extract and sulfasalazine on superoxide dismutase (SOD) activity in colon of mice with acetic acid-induced colitis, compared with normal control (NC) and acetic acid control (AAC) groups. Data are expressed as means \pm SEM ($n \geq 3$). ### $p < 0.001$ acetic acid control vs. normal control; ^{ns} $p > 0.05$, ^{**} $p < 0.01$, and ^{***} $p < 0.001$ vs. acetic acid control

According to Kruidenier *et al.* [48], SOD has powerful anti-inflammatory activity and is highly effective in the treatment of colonic inflammation in experimental colitis by reducing ROS generation and oxidative stress. Hence, *P. argentea* which increased the colon SOD activity levels during ulcerative colitis can be used as a powerful anti-colitic and anti-inflammatory agent and can be used, after further testing as potent chemotherapeutic agent.

Determination of CAT activity

SOD and CAT are involved in the antioxidant defensive mechanisms of cells. SOD catalyzes the dismutation of superoxide to hydrogen peroxide and molecular oxygen, and CAT then converts the hydrogen peroxide to water and molecular oxygen [49,50]. In the present work, the level of CAT activity assessed in all experimental animals is displayed in Fig. 9. Results reveal that activity of CAT significantly ($p < 0.001$) declined in acetic acid-treated animals as compared to normal control ones, whereas treatment with PAME and SASP significantly predominates the levels of CAT activity to normal levels as compared to colitic control animals. Based on the CAT activity levels, we suggest that *P. argentea* extract at 50 mg/kg showed no effect on catalase activity as compared to colitis control group.

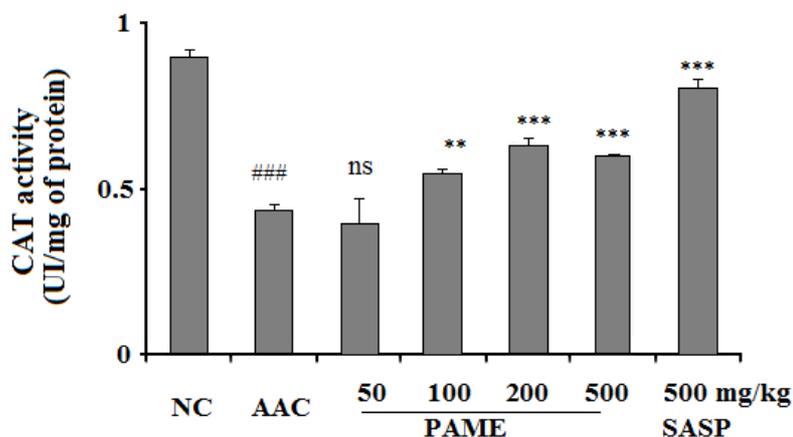


Figure 9. Effects of different doses of *P. argentea* methanolic extract and sulfasalazine on catalase (CAT) activity in colon of mice with acetic acid-induced colitis, compared with normal control (NC) and acetic acid control (AAC) groups. Data are expressed as means \pm SEM ($n \geq 3$). ### $p < 0.001$ acetic acid control vs. normal control; ^{ns} $p > 0.05$, ^{**} $p < 0.01$ and ^{***} $p < 0.001$ vs. acetic acid control

The inflammatory response initiated by acetic acid increases production of ROS which in turn up-regulates hydroxyl radicals and peroxide exacerbation leading to mucosal erosion, distortion, and loss of crypts [51]. Antioxidant enzymes play a vital role in scavenging the free radicals. Therefore, and according to our results, *P. argentea* was able to reduce the ulcerative colitis and the inflammatory effect by activating the antioxidant enzymes and scavenging the free radicals.

CONCLUSION

In conclusion, our results demonstrated that induction of mice colitis by acetic acid for a short time induces hematological alterations, disturbances in the oxidant/antioxidant balance and increases the inflammatory markers. Treatment with PAME has significantly decreased colonic damage, DAI, colon weight, CRP, ESR, and colonic

MDA levels, and enhanced colon length, FBC, colonic GSH levels, colonic SOD and CAT activities. These findings indicate that *P. argentea* has a remarkable anti-inflammatory effect associated with its greater antioxidant activity. Moreover, these assays indicate that this plant extract is a source of bioactive compounds which may be useful therapeutic agents which may be used for treating inflammatory diseases such as ulcerative colitis and minimizing or preventing pathological damage caused by free radicals. However, the components responsible for these antioxidant and anti-inflammatory activities could be one or more compounds isolated from this plant by Braca and colleagues [52]. Therefore, further investigation is needed to examine the effect of each isolated compound present in the plant extracts, and to elucidate the definitive mechanisms of its therapeutic activities.

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