

RESEARCH ARTICLE

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Beneficial effect of *Amorphophallus paeoniifolius* tuber on experimental ulcerative colitis in rats

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ABSTRACT

Context: The tuber of *Amorphophallus paeoniifolius* (Dennst.) Nicolson (Araceae), commonly called Suran or Jimmikan, has high medicinal value and is used ethnomedicinally for the treatment of different gastrointestinal and inflammatory disorders.

Objective: The present study evaluated the effects of extracts of *Amorphophallus paeoniifolius* tubers on acetic acid-induced ulcerative colitis (UC) in rats.

Materials and methods: Wistar rats were orally administered methanol extract (APME) or aqueous extract (APAE) (250 and 500 mg/kg) or standard drug, prednisolone (PRDS) (4 mg/kg) for 7 days. On 6th day of treatment, UC was induced by transrectal instillation of 4% acetic acid (AA) and after 48 h colitis was assessed by measuring colitis parameters, biochemical estimations and histology of colon.

Results: APME or APAE pretreatment significantly ($p < .05-.001$) prevented AA-induced reduction in body weight and increase in colitis parameters viz. stool consistency, colon weight/length ratio and ulcer score, area and index. Extracts treatment attenuated ($p < .001$) increase in alkaline phosphatase and lactate dehydrogenase in serum and myeloperoxidase activity and cytokines in colon tissue due to AA administration. Extracts treatment prevented AA-induced elevation in lipid peroxidation and decline in activities of superoxide dismutase and catalase and reduced-glutathione content ($p < .05-.001$) along with histopathological alterations. PRDS also showed similar ameliorative effect on colitis.

Discussion and conclusion: APME and APAE showed a preventive effect on UC, and ameliorated inflammation and oxidative damage in colon. The effects may be attributed to presence of phytochemicals, betulinic acid, β -sitosterol, and glucomannan. In conclusion, the tuber of *Amorphophallus paeoniifolius* exhibited an anticolitic effect through anti-inflammatory and antioxidant action.

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Suran; acetic acid; antioxidant; anti-inflammatory; betulinic acid

Introduction

Ulcerative colitis (UC) is a chronic inflammatory bowel disease (IBD). It superficially affects the mucosal layer of the intestine and mainly occurs in colon and rectum (Abraham & Cho 2009). The symptoms include diarrhea, blood in the stool, abdominal pain, weight loss, loss of appetite, nutrient deficiencies, fever and anemia (Head & Jurenka 2003). Excessive inflammation and oxidative stress play an important role in the pathogenesis of UC (MacDonald & Murch 1994; Tahan et al. 2011). UC is characterized by the migration of neutrophils, basophils and other leukocytes to the mucosal membranes and the superficial ulcers (Reiff & Kelly 2010). This process leads to release of various inflammatory mediators like cytokines and arachidonic acid metabolites along with free radicals resulting in oxidative damage to the colonic tissue (Grisham 1994).

The conventional therapy for UC includes aminosaliclates, corticosteroids, antibiotics, and immunomodulators. Despite their effectiveness, these drugs on long-term use pose side effects and compromise the quality of life of patients. The side effects include pancreatitis, nephritis, hepatitis, male infertility, fever,

rashes, arthralgia, fluid retention, weight gain, risk of immunosuppression, mood swings, cataracts, osteoporosis, myopathy, adrenal insufficiency, etc. (Head & Jurenka 2003). In recent time, herbal medicines have gained popularity for the treatment of UC and shown promising effects in the clinical studies. Among herbal drugs, medical plants of food value can be the best option for the treatment of gastrointestinal diseases because they provide the therapeutic benefit while they are consumed as food and source of nutrition.

Amorphophallus paeoniifolius (Dennst.) Nicolson (Araceae) or Elephant foot yam is a crop of South East Asian origin, commonly known as Suran or Jimmikan in India. It is an important constituent of many Ayurvedic preparations (Ayurvedic Formulary of India 2000). The tuber of this plant has high medicinal value and consumed by many people as a food. Pharmacologically, it exhibited anti-inflammatory (De et al. 2010), analgesic (Shilpi et al. 2005), CNS depressant (Das et al. 2009), cytotoxic (Angayarkanni et al. 2007), antibacterial and antifungal (Khan et al. 2008) activities in experimental studies. The tuber contains phytochemicals like β -sitosterol (Srivastava et al. 2014), lupeol (Khare 2007),

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quercetin, gallic acid (Nataraj et al. 2009; Nataraj et al. 2012), and betulinic acid (Tandan & Sharma 2013).

Traditionally, the tuber of *Amorphophallus paeoniifolius* is popularly used for the correction of several ailments like elephantiasis, tumors, hemorrhages, cough, bronchitis, asthma, etc. The tuber has got remarkable effects on gastrointestinal system and corrects various abnormalities viz. hemorrhoids, vomiting, anorexia, dyspepsia, flatulence, colic, constipation, hepatopathy, etc. (Ayurvedic Formulary of India 2000; Nair 1993; Dey et al. 2012). In ethnomedicinal practices, it is consumed for the treatment of piles (hemorrhoids), abdominal pain, and constipation and as stomachic by tribes of Wayanad district, Kerala (Rahman et al. 2013; Devi Prasad et al. 2013).

In view of the myriad of actions of tuber of *Amorphophallus paeoniifolius* on gastrointestinal tract, it was thought worthwhile to study its effect on UC, an inflammatory bowel disorder. Thus, the present study demonstrates the effect of *Amorphophallus paeoniifolius* tuber on AA-induced UC in rats.

Materials and methods

Chemicals and drugs

beta-Sitosterol and betulinic acid standards (Cayman Chemical, USA) were purchased from Genetix Biotech Asia Pvt. Ltd, New Delhi, India. Thiobarbituric acid, reduced glutathione, 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) and quercetin were procured from Sigma-Aldrich, USA. Prednisolone was obtained as gift sample from Macleods Pharmaceutical Ltd., Mumbai, India. Cytokine ELISA kits (Krishgen Biosystems, USA) were procured from local scientific supplier. All other chemicals were of highest purity grade.

Collection and authentication of the tuber

The tubers of *Amorphophallus paeoniifolius* were collected from the local market of Gwalior in December 2011 and identified by Dr. N. K. Pandey, Taxonomist of the Institute. A voucher specimen No. 5-4/10-11/NRIASHRD/Tech/Survey/1611 was deposited in the herbarium of the Institute.

Preparation of extracts and phytochemical screening

The tubers were chopped into thin pieces, shade dried and coarsely powdered. The powdered tuber was extracted with methanol in Soxhlet extractor. The marc was finally macerated with distilled water to obtain aqueous extract. The extracts were dried in a rotary evaporator and stored in desiccator for further use. The percent yield of methanol (APME) and aqueous (APAE) extract was 9.48% w/w and 6.16% w/w, respectively.

Preliminary phytochemical screening (Khandelwal 2006) of APME and APAE revealed presence of carbohydrates, proteins, alkaloids, flavonoids, sterols, phenolic compounds and tannins while glycosides and saponins were found absent.

Quantitative estimation of phytoconstituents

The total glucomannan content of APME and APAE was determined as described previously by Chua et al. (2012) and found to be 1.13 and 9.04 g of glucomannan per 100 g of extract, respectively. The total phenolic content (TPC) of APME and APAE was determined spectrometrically (Singleton et al. 1999)

and found to be 73.1 mg and 141.5 mg tannic acid equivalents (TAE)/g of extract, respectively. The total flavonoid content (TFC) was measured by colorimetric assay (Marinova et al. 2005) and found to be 92.77 mg and 75.47 mg quercetin equivalents/g of extract, respectively.

Estimation of betulinic acid in APME

Betulinic acid, an important constituent of tuber was estimated in APME by high performance liquid chromatography (HPLC) at Natural Remedies Pvt. Ltd., Bangalore, Karnataka, India as described previously (Dey et al. 2016). The HPLC system (Shimadzu Corporation, Japan) having Phenomenex-Luna C-18(2) column and photo diode array detector was used. Standard betulinic acid (Natural Remedy, India, Percent purity $\geq 95\%$) (0.2 mg/mL) or APME (20 mg/mL) was prepared in HPLC grade methanol and eluted in mobile phase consisting of potassium dihydrogen orthophosphate buffer and acetonitrile in the proportion of 85:15. The wavelength, flow rate and injection volume were 205 nm, 1.5 mL/min and 20 μ L, respectively. The chromatograms were recorded. The amount of betulinic acid was estimated by the following formula.

Amount of betulinic acid = [Area of the sample/Area of the standard] $\times$ [Weight of the standard (mg)/Standard dilution (mL)] $\times$ [Sample dilution (mL)/Weight of the sample (mg)] $\times$ Purity of the standard (%)

Fractionation and isolation of APME

The methanol extract (25 g) was chromatographed over silica gel (60–100 mesh) using benzene and benzene-ethyl acetate (19:1; 9:1; 4:1; 1:1). Fractions of 200 mL were collected and evaluated by thin layer chromatography (TLC). Fraction no. 9–22 eluted with benzene (v/v, 100%) showed similar profile on TLC and hence combined. This combined fraction gave a white solid (compound 1, 17 mg) on crystallization using *n*-hexane. Further elution with benzene-ethyl acetate (v/v, 4:1) gave 32 fractions (fraction no. 102–134) with similar profile on TLC and hence combined. This mixture was rechromatographed over silica gel (100–200 mesh) and elution with benzene-ethyl acetate (v/v, 4:1) offered compound 2 (19 mg). Compounds 1 and 2 were identified by their phytochemical tests, melting point, TLC and IR spectral analysis (JASCO FT/IR-4100 Type A).

Animals

Healthy adult male Wistar rats (8–10 weeks age and 220–250 g weight) were used for the study. The animals were housed at standard experimental conditions of temperature ($25 \pm 1^\circ\text{C}$) with relative humidity $50 \pm 5\%$ under 12 h light:dark cycle. They were fed standard rodent chow (Ashirwad brand, Chandigarh, India) and water *ad libitum*. The experiments were performed in accordance with the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) after seeking approval of the Institutional Animals Ethical Committee (IAEC) (Proposal No. NRIASHRD-GWL/IAEC/2013/01).

Acute toxicity study

Acute oral toxicity study was carried out as per Organization for Economic Co-operation and Development (OECD) 423 guideline (OECD 2001). The overnight fasted rats ($n=3$) were orally

administered APME and APAE in the limit dose of 2000 mg/kg and observed continuously for behavioral, neurological and autonomic profiles for 2 h and after a period of 24, 72 h and thereafter up to 14 days for any lethality, moribund state or death. The limit test was repeated in another group of rats ($n=3$) for confirmation and toxic class of LD₅₀ determination. Acute oral toxicity study revealed no toxicity of any nature or moribund stage due to APME or APAE treatment. This indicated that non-observable adverse effect dose level (NOAEL) is more than 2000 mg/kg and approximate LD₅₀ is greater than 2500 mg/kg.

Induction of ulcerative colitis

UC was induced as per previously described method (Fabia et al. 1992; Al-Rejaie et al. 2013). The rats were fasted for 16 h with free access to water. Under light ether anesthesia, the rats were transrectally administered 2 mL of 4% AA solution using soft pediatric catheter, which was advanced 6 cm from anus. Rats were in trendelenburg position during this process and kept for 30 s in head down position to prevent leakage. After this, AA was washed with excess of saline. After 48 h the animals were sacrificed and colitis was confirmed.

Grouping and treatments

The rats were divided into seven groups (six per group) as follows:

Group I: Normal control (NC) rats received 0.9% normal saline, transrectally

Group II: Experimental control (EC) received vehicle (1% Tween 80), 5 mL/kg

Group III: Reference standard group received Prednisolone (PRDS, 4 mg/kg)

Groups IV–V: APME (250 and 500 mg/kg) treated groups

Groups VI–VII: APAE (250 and 500 mg/kg) treated groups

The rats of group II to VII were pretreated orally with vehicle, PRDS and extracts for seven consecutive days and UC was induced on the 6th day of treatment. APME or APAE was used in the doses of 250 and 500 mg/kg (one tenth of the approximate LD₅₀) as mentioned in other previous studies (Shilpi et al. 2005; Nataraj et al. 2012). The dose of prednisolone was selected as per previous reports (Biradar et al. 2011).

After 48 h of AA administration i.e. on 8th day, blood (~1.5 mL) was collected through retro-orbital plexus (Parasuraman et al. 2010) from overnight fasted rats for the estimation of various biochemical parameters. The animals were sacrificed by deep ether anesthesia and the colon tissue, approx. 9 cm in length and 3 cm proximal to the anus, was excised, opened longitudinally and washed in phosphate buffered saline (pH 7.4). The specimens were weighed and colitis parameters were evaluated. The ratio of wet tissue weight to length (w/L) of the colon was estimated in order to evaluate the intensity of the edema (Appleyard & Wallace 1995). The tissues were mounted on a plain paper and the inflammation was noted as macroscopic ulcer score as described previously by Morris et al. (1989). The scoring was done as – Score 0 = no ulcer; Score 1 = mucosal erythema only; Score 2 = mild mucosal edema, slight bleeding or slight erosion; Score 3 = moderate edema, bleeding ulcer or erosions; Score 4 = severe ulceration, erosion, edema and tissue necrosis. The photographs of the mounted tissue were captured. The ulcer area and total area of the mounted colon were measured as described by Minaiyan et al. (2011). The ulcer index was

calculated as follows:

$$\text{Ulcer index} = \frac{\text{Ulcer area of colon (sq. mm)}}{\text{Total area of colon (sq. mm)}}$$

A small cross section of colon was fixed in 10% formaldehyde solution for histology and the remaining tissue was stored at –20 °C until estimation of biochemical parameters.

Measurement of relative body weight (RBW)

The body weight of each rat was measured during the experimental period, once before the treatment and every day during the treatment. The RBW of each rat was then calculated as follows:

$$\text{RBW} = \frac{\text{ABT(g)}}{\text{IB(g)}} \times 100$$

where, ABT is the absolute body weight at one time interval and IB is the weight of rat on the beginning of the treatment.

Evaluation of stool consistency

Stool consistency was measured on 7th day of the treatment as described previously (Thippeswamy et al. 2011). The animals were kept in individual cages and score was recorded. The scoring was done as – Score 0 = Normal; Score 2 = Loose; Score 4 = Diarrhea.

Estimation of biochemical parameters

Alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) were estimated on NANOLAB 240® clinical chemistry analyzer (Trivitron Healthcare Pvt. Ltd, Chennai, India). The piece of colon isolated above was washed thoroughly with ice-cold 0.1 M phosphate buffered saline (pH 7.4). It was blotted dry and homogenized in 1.15% KCl to prepare a 10% w/v suspension. This suspension was centrifuged at 16,000 × g for 1 h in a cooling centrifuge at 0 °C. The supernatant was then employed for further assessment of myeloperoxidase (MPO), inflammatory cytokines, lipid peroxidation (LPO), enzyme activities and reduced glutathione (GSH) content. MPO activity in colon tissue was estimated by the method described previously (Krawisz et al. 1984). One unit of MPO activity is defined as that degrading 1 μmol of peroxide per minute at 25 °C. LPO was assessed by a previously reported method (Jamal & Smith 1985) with some modifications and is expressed as nM MDA/g tissue. The activities of superoxide dismutase (SOD) (Marklund & Marklund 1974) and catalase (CAT) (Aebi 1984) were assessed as described previously and are expressed as units/g of tissue. GSH content was estimated according to Ellman (1959) and is expressed as μM/mg tissue. The inflammatory cytokines IL-1β and IL-6 were measured using ELISA kits as per manufacturer's protocol. The results are expressed as pg/mg tissue.

Histology of colon

A small portion of colon specimens was fixed in 10% neutral buffered formalin. The tissue was further processed by conventional method to obtain thin sections of 5 μm thickness which were subsequently stained with hematoxylin and eosin. The slides were examined under microscope (Olympus, Japan) for pathological changes. A decision analysis was carried out to describe

the severity of lesions on an arbitrary scale ranging from – [Nil]; + minimal [10–20%]; ++ mild [20–30%]; +++ moderate [30–40%]; ++++ severe [40–50%]) in H&E stained histopathological sections and a histomorphological scale for evaluating ulcerative colitis was assigned as shown in Table 1 as compared to colon tissue of normal animal. Scoring of rat colonic lesions in various groups was performed in a blinded fashion based on this histomorphological scale for evaluation effect of various treatments compared to normal control and reference standard. Maximum total score for the best possible outcome (e.g. normal colon tissue) was 24.

Statistical analysis

The data were analyzed using GraphPad Prism version 4.00 for Windows (GraphPad Software, La Jolla, CA). Parametric data were analyzed by one-way ANOVA followed by Tukey's multiple comparison *post hoc* test, while non-parametric data (scores) were analyzed by Kruskal–Wallis test followed by *post hoc* Dunn's multiple comparison test. A statistical difference of $p < .05$ was considered significant in all cases.

Results

Estimation of betulinic acid

HPLC analysis revealed the presence of betulinic acid in APME. The peaks of standard betulinic acid and APME solution are shown in Figure 1(A) and (B), respectively. The estimated amount of betulinic acid was found to be 0.08% (w/w).

Compound isolated

Methanol extract of *Amorphophallus paeoniifolius* tuber yielded two compounds. Both compound 1 and 2 offered positive test for sterols and triterpenoids. On TLC, compound 1 showed R_f of 0.45 using toluene: ethyl acetate (7:3) and compound 2 showed R_f of 0.288 using toluene: ethyl acetate (8:2) as mobile phase. Melting point of compound 1 and 2 were found 136 °C and 296 °C. IR (KBr) spectral analysis of compound 1 showed $\nu_{\max}$ cm^{-1} : 3418.21, 3291.89, 2938.02, 2867.63, 1666.2, 1639.2, 1463.71, 1379.82, 1061.62, 883.23, 801.28 and compound 2 showed $\nu_{\max}$ cm^{-1} : 3695.91, 2962.13, 2918.73, 2850.27, 2359.48, 1709.59, 1411.64, 1261.22, 1095.37, 1024.02 and 804.17. The comparison of the phytochemical, physical and spectral data obtained from literature values (Ramasamy & Saraswathy 2014; Ahmed et al. 2013), revealed compound 1 as β -sitosterol and compound 2 as betulinic acid (Figure 2). The identification of compounds was further confirmed by comparison of their R_f with standard β -sitosterol and betulinic acid.

Acute toxicity study

Acute oral toxicity studies revealed no lethality or any toxic reactions or moribund state up to the end of the study period. APME and APAE were safe up to a dose level of 2000 mg/kg of body weight (limit test) and the observed LD_{50} for oral administration of the extracts was more than 2500 mg/kg.

Effect on relative body weight (RBW)

AA administration in EC group caused significant decrease in the RBW as compared to NC group ($p < .001$) which was significantly attenuated by pretreatment with APME or APAE (250 and 500 mg/kg) ($p < .001$ in all cases). PRDS (4 mg/kg) showed significant ($p < .001$) attenuation of same compared to EC group (Table 2).

Effect on stool consistency

AA administration in EC group increased the stool consistency score as indicated by diarrhea as compared to NC group ($p < .001$). This increase was significantly reduced by pretreatment with APME or APAE at 500 mg/kg ($p < .001$, $p < .01$, respectively) or PRDS (4 mg/kg) ($p < .01$) compared to EC group (Table 2). The lower dose of APME or APAE (250 mg/kg) was found ineffective ($p > .05$) when compared to EC group.

Effect on colon weight/length (w/L) ratio

AA administration in EC group increased the colon w/L ratio as compared to NC group ($p < .001$). Pretreatment with APME (250 and 500 mg/kg) ($p < .001$ in both cases) or APAE (500 mg/kg) ($p < .01$) or PRDS (4 mg/kg) ($p < .001$) significantly decreased colon w/L ratio while APAE at 250 mg/kg did not significantly ($p > .05$) affect the same compared to EC group (Table 2).

Effect on ulcer score, ulcer area and ulcer index

AA administration increased ulcer score ($p < .001$), ulcer area ($p < .001$) and ulcer index ($p < .001$) in EC group as compared to NC group. Pretreatment with APME or APAE at 500 mg/kg caused significant reduction in ulcer score ($p < .05$ – $.01$ wherever applicable) compared to EC group while the lower dose (250 mg/kg) was ineffective ($p > .05$). Tuber extracts at 250 and 500 mg/kg decreased the ulcer area ($p < .01$ – $p < .001$, wherever applicable) and ulcer index ($p < .001$ in all cases) compared to EC group. PRDS (4 mg/kg) treatment also caused significant reduction in ulcer score ($p < .05$), ulcer area ($p < .001$) and ulcer index ($p < .001$) compared to EC group (Table 2).

The macroscopic observations of colon showed that AA administration caused mucosal damage as indicated by marked

Table 1. Histomorphological scale.

Lesions/Parameters	Severity of lesions on the basis of % area of lesions/microscopic field	Histomorphological Score
Edema/necrosis/inflammation congestion or hemorrhage/thickened mucosa/degenerative changes/fibrosis	Absent/not seen (0%) = –	4
	Minimal (5–10%) = +	3
	Mild (10–25%) = ++	2
	Moderate (25–50%) = +++	1
	Severe (more than 50%) = ++++	0

Maximum total score for the best possible outcome was 24 (4 × 6 parameters/lesions).

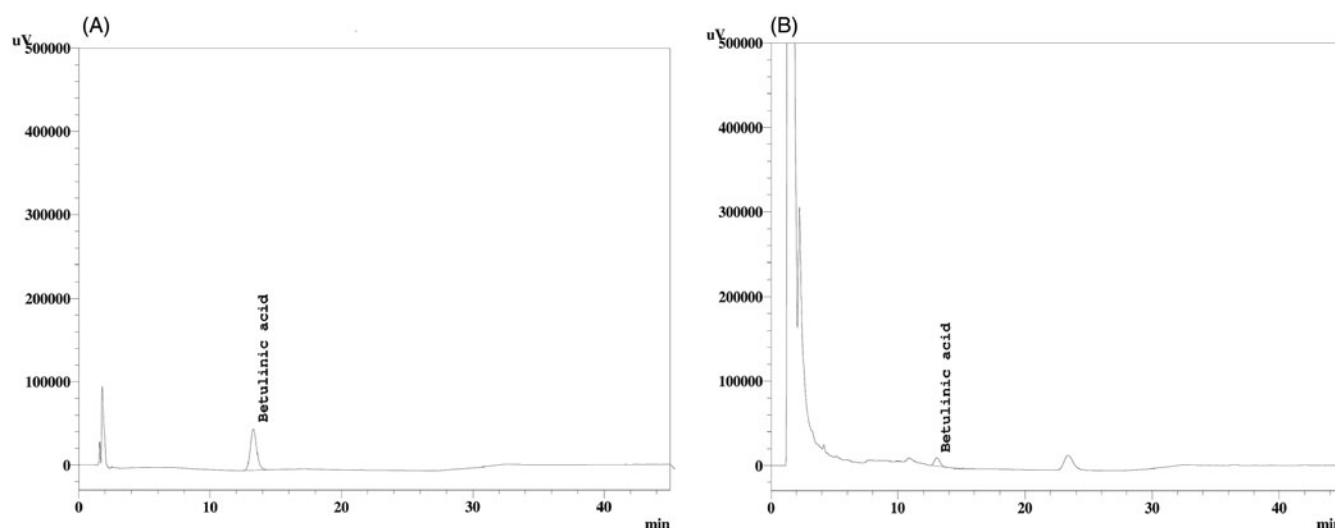


Figure 1. HPLC of APME. The HPLC chromatogram of betulinic acid (standard) (A) and in APME solution (B).

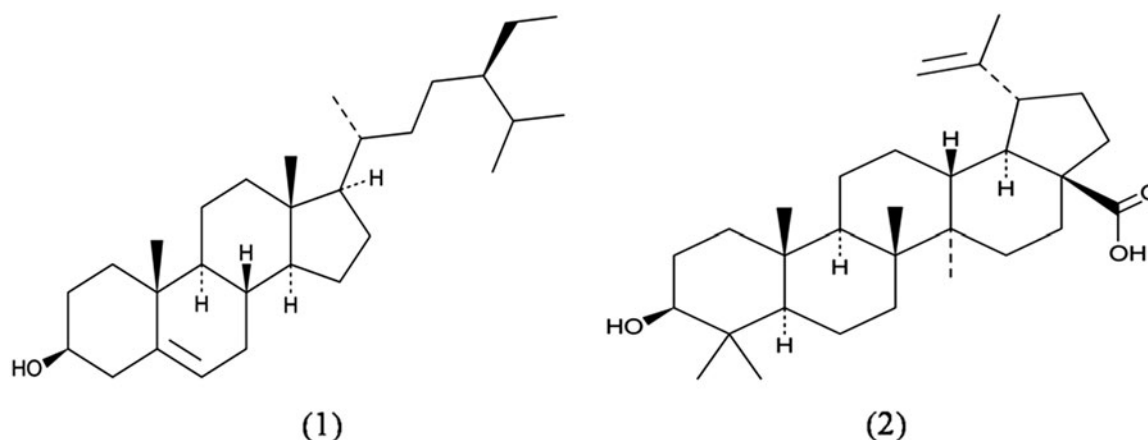


Figure 2. Structures of compounds 1–2. 1 (β -sitosterol), 2 (Betulinic acid).

Table 2. Effect of APME and APAE on relative body weight and colitis parameters.

Groups	Dose (mg/kg)	Relative body weight (%)	Stool consistency (Score)	Colon weight/length ratio (mg/cm)	Macroscopic ulcer score	Ulcer area (mm ²)	Ulcer index
NC		101.40 $\pm$ 0.45	0.00 $\pm$ 0.0	128.63 $\pm$ 2.79	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0
EC		88.44 $\pm$ 0.43 ^a	3.50 $\pm$ 0.22 ^a	288.48 $\pm$ 15.37 ^a	3.67 $\pm$ 0.21 ^a	662.33 $\pm$ 27.59 ^a	0.68 $\pm$ 0.02 ^a
PRDS	4	93.15 $\pm$ 0.48 ^d	0.83 $\pm$ 0.31 ^c	171.21 $\pm$ 11.0 ^d	1.67 $\pm$ 0.21 ^b	334.57 $\pm$ 36.41 ^d	0.34 $\pm$ 0.02 ^d
APME	250	94.54 $\pm$ 0.83 ^d	1.67 $\pm$ 0.21	186.61 $\pm$ 19.46 ^d	1.83 $\pm$ 0.17	368.39 $\pm$ 37.31 ^d	0.30 $\pm$ 0.04 ^d
APME	500	95.89 $\pm$ 0.74 ^d	0.50 $\pm$ 0.22 ^d	177.76 $\pm$ 13.74 ^d	1.50 $\pm$ 0.22 ^c	237.11 $\pm$ 50.93 ^d	0.23 $\pm$ 0.05 ^d
APAE	250	92.11 $\pm$ 0.46 ^d	1.83 $\pm$ 0.31	232.83 $\pm$ 7.96	2.0 $\pm$ 0.26	451.75 $\pm$ 63.86 ^c	0.36 $\pm$ 0.03 ^d
APAE	500	94.69 $\pm$ 0.24 ^d	0.83 $\pm$ 0.17 ^c	211.09 $\pm$ 12.61 ^c	1.67 $\pm$ 0.2d ^b	327.44 $\pm$ 25.77 ^d	0.33 $\pm$ 0.03 ^d

Values are mean $\pm$ SEM. ^a p < .001 when compared to NC, ^b p < .05, ^c p < .01, ^d p < .001 when compared to EC.

ulceration and inflammation in colonic tissue compared to NC group which showed normal morphology of the tissue. Pretreatment with APME or APAE in the dose of 250 and 500 mg/kg exhibited marked decrease in mucosal damage, ulceration and inflammation as compared to EC group. PRDS (4 mg/kg) treatment also showed prominent decrease in ulceration and inflammatory changes compared to EC group. Figure 3 depicts the photographs of microscopic observations after various treatments.

Effect on ALP, LDH and colonic MPO activity

In EC group, AA administration increased the serum levels of ALP (p < .001) and LDH (p < .001), and colonic MPO activity

(p < .001) compared to NC group. Pretreatment with APME or APAE (250 and 500 mg/kg) significantly attenuated the elevation in levels of ALP, LDH and MPO (p < .001 in all cases). PRDS (4 mg/kg) showed significant attenuation of increase in ALP (p < .001), LDH (p < .001) and MPO activity (p < .001) compared to EC group (Table 3).

Effect on oxidative stress parameters in colon tissue

In EC group, AA caused oxidative stress in colonic tissues as indicated by increased LPO levels (p < .001), decreased (p < .001) activities of SOD and CAT and GSH levels (p < .001). Pretreatment with APME (250 and 500 mg/kg) or APAE (500 mg/kg) significantly decreased elevation in LPO levels

($p < .001$ in all cases), prevented the reductions in the activities of SOD ($p < .001$ in all cases) and CAT ($p < 0.001$ in all cases) and attenuated decrease in GSH content ($p < .001$ in all cases) due to AA administration. The lower dose of APAE (250 mg/kg) did not influence ($p > .05$) LPO and the activities of antioxidant enzymes and GSH. PRDS (4 mg/kg) significantly attenuated

elevation in LPO ($p < .05$) and decrease in CAT activity ($p < .01$) while SOD and GSH remained unaffected ($p > .05$) compared to EC group (Table 3).

Effect on inflammatory cytokines in colon tissue

In EC group, AA caused significant increase in IL-1 β ($p < .001$) and IL-6 ($p < .001$) compared to NC group. Pretreatment with APME (500 mg/kg) or APAE (500 mg/kg) significantly attenuated elevation in IL-1 β ($p < .01$ and $p < .05$, respectively) and IL-6 ($p < .01$ and $p < .05$, respectively) compared to EC group. The lower dose of APME or APAE (250 mg/kg) did not influence ($p > .05$) any of the cytokine compared to EC group. PRDS also showed decrease in IL-1 β and IL-6 ($p < .001$ in all cases) compared to EC group (Figure 4).

Effect on histology of colon tissue

Kruskal–Wallis analysis of total histomorphological score and the photomicrographs illustrated that AA administration (Figure 5(B)) caused significant ($p < .001$) alterations in colon tissue as indicated by severe thickened mucosa, infiltration of inflammatory cells, sloughing of mucosa, tissue necrosis and mild hemorrhage in EC group as compared to colon of normal rats (NC) (Figure 5(A)) which showed normal architecture. Pretreatment with APME prevented ($p < .05$) colonic tissue damage due to AA administration as indicated by lesser degree of tissue necrosis and hemorrhage (Figure 5(D)) compared to EC group (Figure 5(B)) while APAE (Figure 5(E)) showed non-significant ($p > .05$) prevention of the damage. PRDS also exhibited ($p < .01$) marked reduction in the damage caused by AA administration (Figure 5(C)). The histological observations quantified as histomorphological score are indicated in Table 4.

Discussion

The present study demonstrates the beneficial effect of the tuber extracts of *Amorphophallus paeoniifolius* against experimental ulcerative colitis in rats. Pretreatment with tuber extracts significantly ameliorated AA-induced colonic mucosal damage, inflammation, oxidative and histological alteration which was confirmed by comparison with standard drug, PRDS.

AA-induced ulcerative colitis is a well-established animal model (Hartmann et al. 2012) where AA causes colonic epithelial lesions, necrosis, and infiltration of neutrophils and macrophages to the damaged colon indicating colonic inflammatory condition (Popov et al. 2006). In the present study, transrectal administration of 4% AA caused induction of UC. Body weight reduction and loss of water and electrolytes in stools are characteristic features of UC (Chung et al. 2007). In the present study, there was marked decrease in relative body weight of animals in colitis group (EC) similar to the results of previous studies (Zeng et al. 2011).

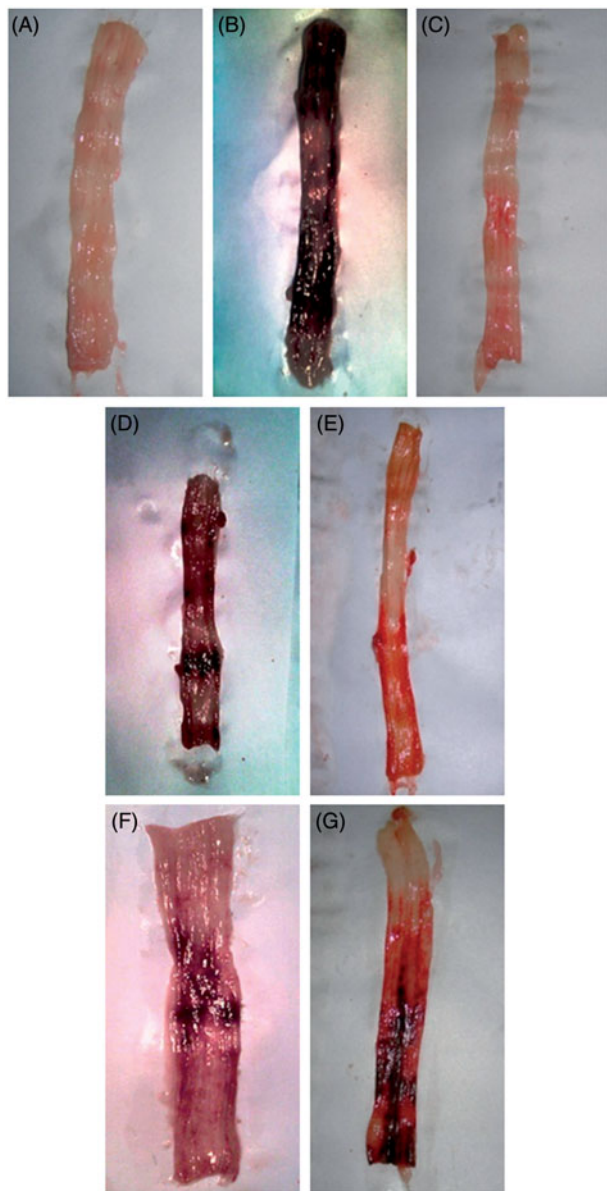


Figure 3. Effects of APME and APAE on macroscopic observation of the colon. The colon from NC rats (A) exhibited normal morphology of colon while the colon from EC rats (B) showed ulceration and inflammation. The colons from PRDS (4 mg/kg) (C), APME (250 and 500 mg/kg) (D and E, respectively), APAE (250 and 500 mg/kg), (F and G, respectively) showed a lesser degree of mucosal damage, ulceration and inflammation compared to B (EC group).

Table 3. Effect of APME and APAE on serum ALP, LDH and colon tissue LPO, SOD, CAT and GSH.

Groups	Dose (mg/kg)	ALP (IU/L)	LDH (IU/L)	MPO (U/mg tissue)	LPO (nM MDA/g tissue)	SOD (U/g tissue)	CAT (U/g tissue)	GSH (μ M GSH/mg tissue)
NC		47.17 $\pm$ 5.04	559.333 $\pm$ 60.21	2.90 $\pm$ 0.32	6.30 $\pm$ 0.70	488.26 $\pm$ 16.52	68.18 $\pm$ 3.33	12.73 $\pm$ 1.10
EC		135.00 $\pm$ 7.99 ^a	2620.67 $\pm$ 108.34 ^a	16.33 $\pm$ 0.54 ^a	16.00 $\pm$ 1.19 ^a	181.13 $\pm$ 19.07 ^a	27.91 $\pm$ 1.47 ^a	2.91 $\pm$ 0.68 ^a
PRDS	4	55.17 $\pm$ 2.52 ^d	1599.33 $\pm$ 54.16 ^d	9.77 $\pm$ 0.30 ^d	9.86 $\pm$ 1.12 ^b	252.65 $\pm$ 17.26	54.01 $\pm$ 4.59 ^c	4.89 $\pm$ 0.62
APME	250	65.67 $\pm$ 3.21 ^d	1779.67 $\pm$ 89.18 ^d	10.30 $\pm$ 0.53 ^d	9.18 $\pm$ 0.62 ^d	324.80 $\pm$ 15.71 ^d	55.85 $\pm$ 4.51 ^d	7.98 $\pm$ 0.50 ^d
APME	500	51.0 $\pm$ 0.52 ^d	1403.41 $\pm$ 74.52 ^d	9.00 $\pm$ 0.54 ^d	7.14 $\pm$ 0.64 ^d	370.23 $\pm$ 30.84 ^d	64.58 $\pm$ 3.8 ^d	8.64 $\pm$ 0.75 ^d
APAE	250	74.0 $\pm$ 8.69 ^d	1854.17 $\pm$ 75.39 ^d	11.76 $\pm$ 0.48 ^d	14.98 $\pm$ 1.08	269.56 $\pm$ 15.68	37.32 $\pm$ 2.02	5.19 $\pm$ 0.70
APAE	500	52.83 $\pm$ 7.90 ^d	1548.17 $\pm$ 103.69 ^d	9.79 $\pm$.91 ^d	8.94 $\pm$ 0.99 ^d	328.17 $\pm$ 28.15 ^d	57.76 $\pm$ 5.11 ^d	8.23 $\pm$ 0.79 ^d

Values are expressed as mean $\pm$ SEM. ^a $p < .001$ when compared to NC, ^b $p < .05$, ^c $p < .01$, ^d $p < .001$ when compared to EC.

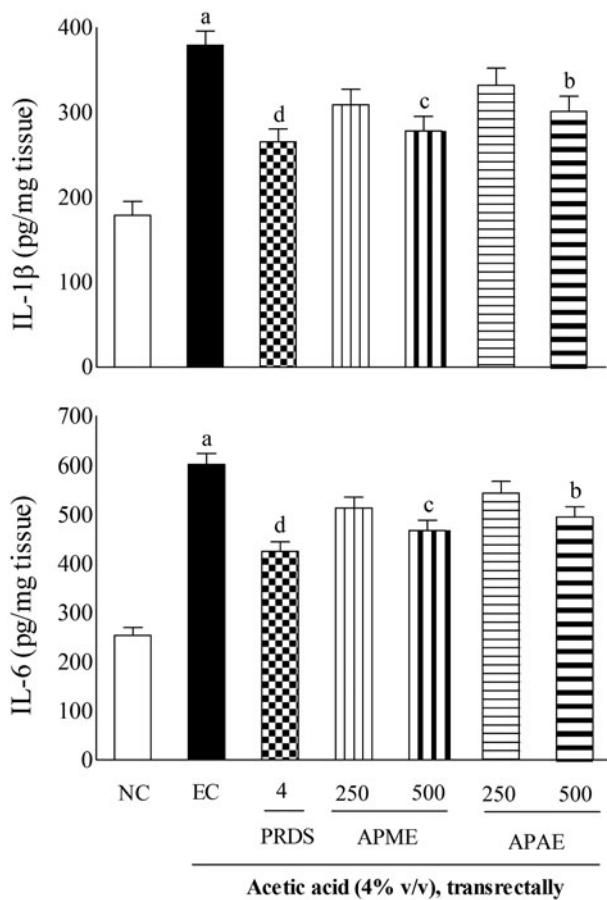


Figure 4. Effects of APME and APAE on inflammatory cytokines. Results are expressed as mean $\pm$ SEM. ^a $p < .001$ when compared to NC, ^b $p < .05$, ^c $p < .01$, ^d $p < 0.001$ when compared to EC.

The body weight reduction of animals is suggestive of their debilitated condition due to colitis. The diarrheal stool consistency was also observed in EC group and confirmed loss of body fluid. Colonic inflammation causes bloody stools and diarrhea (Kumar et al. 2014) contributing to body fluid loss and debility and corroborates the findings. The treatment with APME or APAE or PRDS showed restoration of the body weight loss and improved stool consistency. The body weight restoration might have occurred due to restoration of metabolism and cellular biosynthesis.

Increased macroscopic colitis score (ulcer score), ulcer area and ulcer index with marked erythema of the colonic wall indicate mucosal damage and ulceration due to AA (Table 2 and Figure 3). The significant increase in wet weight of the colon and colon w/L ratio reflect the severity of UC, degree of inflammation, swelling, thickening of intestinal walls due to ulceration (Thippeswamy et al. 2011; Rachmilewitz et al. 1989) and suggest the inflammatory nature of AA-induced UC. This is further evident from histopathological observations (Table 4 and Figure 5) and confirmed the AA-induced inflammatory changes and necrosis of tissue as mentioned above. Pretreatment with APME or APAE or PRDS treatment decreased the increase in ulcer score, ulcer area and ulcer index due to AA administration along with reduction in colon weight and colon w/L ratio. This suggests the beneficial action of the extracts against ulcerative changes similar to PRDS. The decrease in colon lesions and ulceration due to APME or APAE or PRDS treatment is evident from macroscopic (Figure 3) as well as from the histopathological observations (Table 4 and Figure 5) of colon tissue, which indicated lesser

degree of inflammatory changes and necrosis after drug treatments.

The biochemical estimations revealed that AA administration increased the levels of ALP, which is in agreement with previous studies (Kumar et al. 2014). ALP is phosphohydrolase enzyme, attached with glycosyl phosphatidyl inositol, anchors to the cell wall and its activity is considered as an important hallmark for inflammation during the UC (Schreiber et al. 1997). The elevated levels of ALP support the inflammatory changes in UC. The observed decrease in ALP due to APME or APAE or PRDS treatment indicates prevention of UC through anti-inflammatory action. LDH is a cytoplasmic enzyme in cells and its extracellular appearance suggests the cell damage, death or tissue injury (Drent et al. 1996). The increased LDH levels due to AA indicate that there was colonic tissue damage. APME or APAE and PRDS treatments attenuated the elevation in LDH levels indicating the prevention of colonic tissue damage.

MPO is an enzyme abundantly found in neutrophils but can also be found in lesser amounts in other types of leukocytes. MPO activity is a marker of tissue neutrophils concentration (Bradley et al. 1982). The influx of neutrophils and macrophages into the bowel wall during inflammation contributes to tissue damage due to the release of MPO. AA administration caused increase in levels of MPO in colon tissue and suggests the neutrophils infiltration and subsequent inflammation (Patil et al. 2012). UC is marked by infiltration of large number of neutrophils into mucosal interstitium (lamina propria), a characteristic feature of inflammatory condition (Reiff & Kelly 2010) and thus supports the present findings. APME or APAE or PRDS treatment significantly decreased the MPO levels. In concordance with elevated MPO activity, the histological findings also revealed the leukocytes infiltration in EC group, which was attenuated in extracts or PRDS treated groups (Table 3 and Figure 5). The inhibition of MPO activity and subsequent leukocytes infiltration substantiate anti-inflammatory role of extracts in preventing UC (De et al. 2010).

Oxidative stress plays a pivotal role in the pathophysiology of UC (Rana et al. 2014) and the involvement of free radicals has been demonstrated in experimental UC (Al-Rejaie et al. 2013; Kumar et al. 2014). During UC, certain pro-inflammatory agents such as leukotriene B₄, platelet-activating factor and immune complexes activate inflammatory phagocytes (neutrophils), which in turn release large amounts of reactive oxygen species (ROS) into the extracellular space (Grisham 1993; Grisham 1994). In the present study, AA administration caused induction of oxidative stress in colon tissue as indicated by increase in LPO levels and decreased activity of SOD and CAT enzymes, and GSH content. APME or APAE treatment significantly attenuated oxidative stress by reducing LPO levels and restoring the changes in SOD and CAT activity and GSH content (Table 3). However, the standard drug – PRDS could only attenuate LPO elevation and decrease in CAT activity without influencing SOD activity and GSH content. The preventive effect of PRDS on UC suggests that the oxidative stress observed in EC is subsequent to activation of neutrophils by inflammatory mediators. The observed attenuation of increase in LPO and decrease in CAT activity by PRDS might be because of inhibitory effect on neutrophils activation as a part of its anti-inflammatory effect. However, why PRDS did not affect SOD and GSH content is difficult to interpret. It is possible that anti-inflammatory effect of PRDS could have partially prevented the production of ROS by neutrophils and while others were still being defended by antioxidant enzymes and GSH. The amelioration of oxidative stress parameters indicates preventive

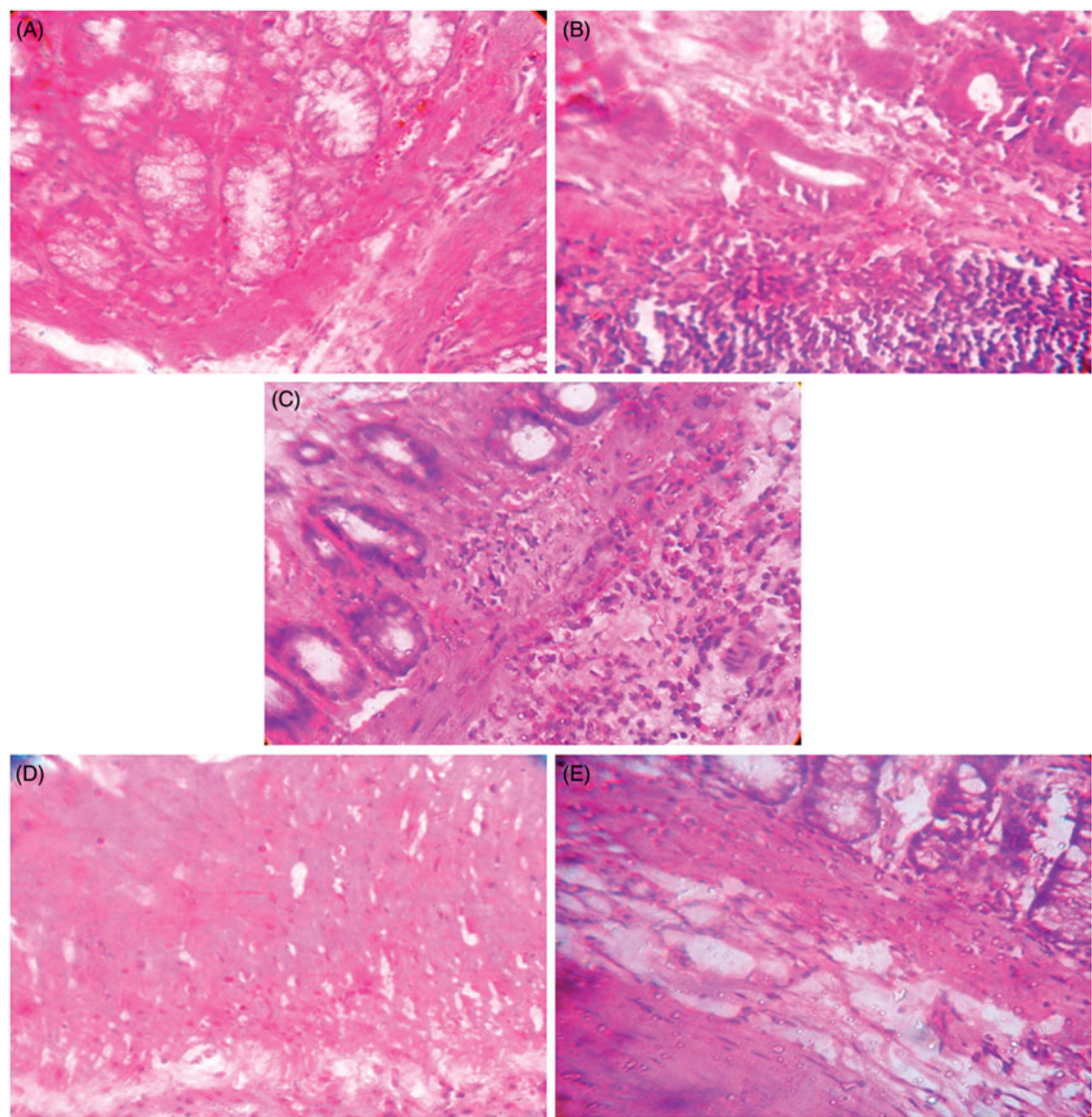


Figure 5. Effect of APME and APAE on histology of colon. Histological sections of colon from saline treated NC rats (A) showing normal architecture. Colon section from acetic acid treated EC rats (B) that received vehicle shows severe thickened mucosa, increased infiltration of inflammatory cells, edema, sloughing of mucosa, tissue necrosis and mild hemorrhage as compared to normal rats (NC). Section of colon from acetic acid treated rat which concurrently received PRDS (4 mg/kg) (C), APME (500 mg/kg) (D) and APAE (500 mg/kg) (E) orally for seven days shows a marked decrease in infiltration of inflammatory cells, edema, sloughing of mucosa, tissue necrosis and hemorrhage and indicates the prevention of the damage compared to vehicle treated EC group. (Type of staining: Hematoxylin and Eosin. Magnification: ×400).

Table 4. Summary of histological changes.

Histological lesions/parameters	NC	EC	PRDS 4 mg/kg	APME 500 mg/kg	APAE 500mg/kg
Thickened mucosa	4.0 ± 0	0.167 ± 0.167	2.50 ± 0.224	2.333 ± 0.211	1.333 ± 0.211
Inflammatory cells infiltration	3.333 ± 0.211	0 ± 0	2.0 ± 0.258	2.0 ± 0.258	2.0 ± 0.258
Hemorrhage	3.833 ± 0.167	1.0 ± 0.258	1.833 ± 0.307	1.0 ± 0.258	1.0 ± 0.258
Edema	4.0 ± 0	0 ± 0	3.0 ± 0.258	2.167 ± 0.167	1.833 ± 0.167
Sloughing of mucosa	4.0 ± 0	0.667 ± 0.211	3.667 ± 0.211	2.667 ± 0.211	1.833 ± 0.167
Necrosis	4.0 ± 0	0 ± 0	2.833 ± 0.1667	2.333 ± 0.211	1.50 ± 0.224
Total histomorphological score	23.17 ± 0.167	1.83 ± 0.477 ^a	15.83 ± 0.872 ^c	13.33 ± 0.494 ^b	9.50 ± 0.50

Values are mean ± SEM. ^a*p* < .001 when compared to NC, ^b*p* < .05, ^c*p* < .01 when compared to EC.

effect of extracts on the oxidative damage. Thus, the beneficial effect of extracts on UC may be accounted to anti-inflammatory and antioxidant action of the extract. Previously, *Embllica officinalis*, *Gingko biloba* and *Dillenia indica* exhibited preventive effect on AA-induced UC owing to their antioxidant and anti-inflammatory nature (Deshmukh et al. 2010; Hartmann et al. 2012; Somani et al. 2014) which further strengthen the results of the present study.

During UC, inflammatory cells chemotaxis leads to release of inflammatory cytokines such as tumor necrosis factor- α , IL-1 β and IL-6 (Sartor 1997). They modulate mucosal immune system and disrupt the epithelial integrity leading to colonic damage. This triggers the pathological response and further worsens the disease condition (Grisham & Yamada 1992). In the present study, elevated colonic levels of IL-1 β and IL-6 in EC group further confirms the inflammatory affliction of colon and justifies

the severity of colonic damage due to AA. This is in concordance with previous studies (Tahan et al. 2011; Al-Rejaie et al. 2013). The diminished levels of cytokines in APME, APAE and Prednisolone pretreated group substantiate the anti-inflammatory effect of extracts and amelioration of colonic inflammation (Figure 4).

The phytochemical screening of the extracts revealed the presence of carbohydrates, proteins, steroids, flavonoids, phenolic compounds and tannins. In the present study, the quantitative estimation of TFC and TPC revealed fair amount of flavonoids and phenolic compounds in the extracts. In affirmation to presence of betulinic acid (Tandan & Sharma 2013), HPLC analysis also revealed presence of betulinic acid (0.08%) (Figure 1). The fractionation and isolation of methanol extract yielded β -sitosterol and betulinic acid and further strengthen the findings. Previously, betulinic acid demonstrated anticolitic effect on 2,4,6-trinitrobenzenesulfonic acid (TNBS)-induced UC in rats through its inhibitory influence on inflammatory mediators and antioxidant activity (Şener et al. 2013). In addition to betulinic acid, β -sitosterol also exhibited inhibitory influence on TNBS-induced UC in mice through inhibition of proinflammatory cytokines and cyclooxygenase (COX-2) enzymes (Lee et al. 2012). Recently β -sitosterol showed preventive effect on dextran sodium sulphate-induced colitis through anti-inflammatory action (Aldini et al. 2014). This evidence suggests that the anticolitic effect of tuber extracts may be due to the presence of betulinic acid and β -sitosterol. Other phenolic compounds like lupeol and quercetin which are present in *Amorphophallus paeoniifolius* tuber which showed preventive effect on UC (Lee et al. 2016; Sotnikova et al. 2013) and supports the anticolitic effect of tuber. The tuber contains nearly 70% of the carbohydrates (Srivastava et al. 2014), and has high content of glucomannan, a water-soluble fiber (Nguyen et al. 2009). In concordance, phytochemical studies revealed the presence of fair amount of glucomannan in APAE and APME. In clinical studies, konjac glucomannan has demonstrated therapeutic effectiveness in inflammatory bowel disease (Suwannaporn et al. 2013) and suggests the contributing role of glucomannan in anticolitic effect of tuber. Hence, the beneficial effect of APME and APAE on UC may be attributed to the presence of betulinic acid, β -sitosterol and glucomannan. However, the pharmacological effectiveness of extracts needs further confirmation with investigations on fractions and isolated compounds, supported with on genetic or protein level studies.

The use of AA as experimental model of UC has main limitation that inflammation produced in this model is not identical to human UC, but it has some histological similarities to human UC including epithelial cell necrosis, decreased mucin production, crypt abscesses and infiltration of large number of neutrophils into mucosal interstitium (MacPherson & Pfeiffer 1978). Furthermore, inflammation and tissue injury observed in Human IBD are results of immunologic activation whereas the inflammation induced by AA is a result of extensive mucosal injury (Yamada & Grisham 1994). Hence, there is a need to check the effect of these extracts in other animal models of ulcerative colitis.

Conclusions

Findings of the present study exhibit a preventive effect of tuber extracts of *Amorphophallus paeoniifolius* against acetic acid induced ulcerative colitis in Wistar rats. The preventive effect could be attributed to anti-inflammatory and antioxidant property of bioactive constituent present in tuber extracts.

The outcome of the study implicates the therapeutic potential of *Amorphophallus paeoniifolius* tuber to be developed as alternative treatment for inflammatory bowel diseases.

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Disclosure statement

The authors declare that there is no conflict of interest regarding the publication of the paper.

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