Musa Acuminata Wastes as New Potential source of Anti-ulcerative Colitis Agents : Pharmacological and Metabolomics Profiles

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Abstract

Musa acuminata is a popular fruit in the world. Non-food parts of the plant have been investigated for their antioxidant and anti-ulcerative colitis activity. Metabolomic approaches was found to be informative as a screening tool. It discovered different metabolites depending on statistical analysis. The antioxidant activity content was measured by colorimetric method. Seventy six investigated metabolites were observed. The identities of some of these markers were confirmed based on their MS^2 fragmentation and NMR spectroscopy. These include: Cinnamic acid and its dimer 2-hydroxy-4-(4-methoxyphenyl)-1H-phenalen-1-one beside Gallic acid and flavonoids; Quercetin, Quercetin-3-*O*-*β*-D-glucoside, Luteolin-7-*O*-*β*-D-glucopyranoside. The leaves, pseudostem and fruit peels extracts were tested for their safety and their antiUlcerative-colitis efficacy in rats. Rats were classified into:Normal, Positive, prednisolone reference group , Musa extracts pretreated groups(250-500mg/kg) for two weeks followed by induction of Ulcerative-colitis by per-rectal infusion of 8% acetic acid. Macroscopic and microscopic examination were done. Inflammatory markers (ANCA, CRP and Ilβ6) were measured in sera. The extracts showed good antioxidant and anti-inflammatory activities as they ameliorated macroscopic and microscopic signs of Ulcerativecolitis and lowered the inflammatory markers compared to untreated group. Musa wastes can be a potential source of bioactive metabolites for industrial use and future employment as promising anti-Ulcerativecolitis food supplements.

Highlights

This research was carried out to investigate the chemical composition, and phytochemicals of some banana by-products; leaves, stem and fruit peels. All samples were extracted by, petroleum ether, dichloromethane, ethylacetate, butanol and water.

- Total phenols, flavonoids and phenylphenalenones for all extracts were identified as well as the sample extracts were evaluated for their antioxidant and anti-ulcer activities. Phenolic compounds isolated from butanol extracts of leaves samples were characterized and identified by Column chromatography.
- Antioxidant assays by $DPPH^{\bullet}$ and $ABTS^{\bullet+}$, reducing power were evaluated. The best IC_{50} values to quench the $DPPH^{\cdot}$ and $ABTS^{\bullet+}$ chelating of these samples extracts were obtained by different extracts. Butanol extracts showed higher antioxidant activity than ethyl acetate extracts of banana leaves.
- This study aimed to characterize Musa waste (leaves, stem and fruit) based on their phenolic composition and study their safety and efficacy in bowel disease “Ulcerative Colitis”.
- This study with its obtained important results formed an added value to agriculture wastes which represent a great problem of contamination by exchange its nature from pullet and factor to an added value by its phytochemical potentially.

1. Introduction

Local body response to continuous tissue irritation by mechanical trauma or recurrent infection or chemical injury lead to inflammatory response in the form of swelling, redness and pain. Inflammation occurs as a defensive mechanism against spread of traumatic insult and subsequent threat to affected organ [1]. Inflammatory diseases can affect the patient’s life quality adversely, which necessitates great care and continuous treatment with specific regimens [2].

Inflammation affects also internal organs as digestive system. The most serious digestive system inflammatory diseases are those of bowel diseases including Ulcerative colitis which affect a considerable range of population around the world. The rate of occurrence of Ulcerative colitis (UC) is very high and is continuously increasing yearly. It affects patients at early adolescence and continues to progress throughout life [3]. One of the well established characteristic of UC is chronic, colonic mucosal inflammation that is usually relapsing and is manifested by episodic attacks of severe abdominal pain that persist for a period of time and is associated with catarrhal or bloody diarrhea [4].

Unfortunately as a whole current medication aren’t always to the expectations and have adverse effects on patient’s health, as treatment of mild or moderate UC is achieved by 5-aminosalicylates or sulfasalazine, while moderate or severe UC are treated by high-dose of oral or intravenous corticosteroid. However primary remission may not be accessed for all patients and consequently corticosteroid dependence or resistance may occur [5], which may end up by total or partial colectomy [6]. That’s why there is need for introduction of supplementary herbal medicines in the regimen of treatment of UC provided that they don’t have evidenced side effects [1]. Musa spp. (bananas) is a good sources of carbohydrates, proteins, other vitamins and minerals. They contain different amino acids like threonine, tryptamine, tryptophan, as well as flavonoids, dopamine, beta-carotene and sterols [7]. Studying the biological activities of banana different parts was carried outin various sudies ; where stem were studied as antidiabetic supplements which improved the level of insulin and reduced blood glucose as well as glycosylated haemoglobin through modulating carbohydrate metabolizing enzymes activity[8, 9],also they have glycaemic effects oweing to their high content of sodium and potassium, fruits were studied as healing and antiulcerative agents, moreover methanolic as well as aqueous extracts of *Musa. paradisiaca* (banana) were reported to produce wound healing in rats [7, 11], peel were studied for their immunomodulatory effects [10]. Additional evidence of the healing activity of Musa is that when ulcer was induced in experimental animals using non- steroidal anti-inflammatory drugs or steroid or histamine, oral administration of banana pulp powder showed marked antiulcerogenic effect[12].

Phytochemical profile studying using a metabolomic approach of Leaves of bananas in previous studied using UPLC–QToF–MS technology, showed that thirty-one compounds were identities of some of these markers based on their MS2 fragmentation. These include quercetin *O*-rhamnoside -*O* hexoside, kaempferol-3-*O*-rutinoside, quercetin-*O*-hexoside, isorhamnetin-*O*-rutinoside and hexadecanoic acid.

Though accessions by soxhlet gave better yield (20.0–60.0%) than by sonication (18.4–23.0%),neither motherland nor methods of extraction had any significant role in the separation process [7].

This research was carried out to investigate the chemical composition, and phytochemicals of some banana by-products; leaves, stem and fruit and study their potential protective effects against colonic inflammatory insult induced by acetic acid in rats, to mimic signs and symptoms of the serious devastating inflammatory bowel disease “Ulcerative Colitis” aiming at introducing a new functional anti-inflammatory food supplement.

2. Material And Methods

Guideline ethics for Plant usage in the phytochemical study

The present study complies with local and national guidelines as permission was obtained for collection of plant material.

Guideline ethics for Experimental animal handling in the in vivo pharmacological study: The study was done in accordance with the guide for care and use of laboratory animals Experiments were performed according to the National Regulations of Animal Welfare and the Institutional Animal Ethical Committee (IAEC),and is reported in accordance with Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines.

Ethical approval

was obtained from National Research Centre ethics committee under number 16/138

2.1. Phytochemical Study

2.1.1. Chemicals

ABTS, DPPH, Trolox and Folin-Ciocalteu reagent (FCR) were purchased from Sigma Aldrich (GmbH). All chemicals and solvents used in this study were supplied by Fisher Scientific UK (Bishop Meadow Road, Loughborough) with high analytical grade.

2.1.2. Plant materials, extraction, isolation and identification

Musa acuminata (MA) waste was collected as (leaves, pseudo-stem and fruit peels) from private farm at Anshase, Giza Governorate. Permission was obtained for collection of plant material. The MA waste was exhaustively extracted three times with 5L of methanol at room temperature, then filtered and concentrated under reduced pressure at 45°C using rotary evaporator. The three crude residues were suspended in 1L water, left overnight and then it was successively partitioned with 0.7L of petroleum ether, chloroform three times, followed by 0.7L ethyl acetate then 0.7L n-butanol three times repeatedly.

Chromatographic procedure for isolation and identification of some phenolics compounds from three kg of powdered leaves were extracted with EtOH (80%) by soaking at room temperature. The combined alcoholic extracts were concentrated under reduced pressure at 45°C, which yield 365 g of residue. The crude residue was suspended into water then overnight partitioned with petroleum ether, methylene chloride, ethyl acetate and n-butanol. The n-butanol fraction was chosen for further purification. The n-butanol fraction (g) was loaded on a column (3.5 cm×150 cm) of Polyamide, and the column was stepwise eluted with water, 30, 50, 70, and 100% methanol at a flow rate of 50 mL/min to yield five sub fractions. 50% and 70% methanol sub-fraction was further purified using a sephadex LH-20 column chromatography and eluted with 50% methanol, to yield 6 compound. The jar of different elution solvent systems were used (BAW 4:2:1 or 30:10:10, acetic acid 15%, and Benzene: MeOH: Acetic Acid 45:8:4). After air-drying, the spots were visualized under UV light [13]. From 50% of alcohol gives five compound flavonoids and phenolics acids and one compound from 70% alcohol that identified by spectral tools data.

2.1.3. Appraising, detecting, identifying and characterizing secondary metabolites.

2.1.3.1. GC/MS of essential oil fruit peels extract.

The volatile oil of waste fresh fruit peels MA was extracted by water distillation method as Solvent-Assisted Flavour Evaporation (SAFE). The homogenate was continuously steam-distilled by diethyl ether (25 mL) extracted in 3 h in a Likens-Nickerson apparatus [14]. The resulted essential oil was separately dehydrated with anhydrous sodium sulphate and kept in deep freezer until GC/MS analysis. The analysis was done in triplicate and the mean values of the oil content (%) were recorded. The components of essential oil in MA were identified by GC/MS analysis instrument stands at the Department of Medicinal and Aromatic Plants Research, National Research Center with the following specifications. Instrument: a TRACE GC Ultra Gas Chromatographs (THERMO Scientific Corp., USA), coupled with a THERMO mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer). The GC/MS system was equipped with a TG-WAX MS column (30 m x 0.25 mm i.d., 0.25 µm film thickness). Analyses were carried out using helium as a carrier gas at a flow rate of 1.0 mL min^{-1} and a split ratio of 1:10 using the following temperature program: 60°C for 1min; rising at 4.0 $^{o}C\ min^{-1}$ to 240°C, and held for one minute. The injector and detector were held at 210°C. Diluted samples (1:9 diethyl ether, v/v) of one µL of the mixtures were always injected. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of m/z 40–450. Most of the compounds were identified using the analytical method: mass spectra (authentic chemicals, Wiley spectral library collection and NSIT library)[15].

2.1.3.2. HPLC & LC/MSMS of secondary metabolites from MA wastes.

a) Quantitative of phenolic compounds from Butanol fractions of three parts of MA were measured using authentic fifteen standards. The HP 1100 -HPLC system (Agilent Technologies, Palo Alto, CA, USA) with an auto-sampler (G1329B), quaternary pump and a diode array detector.

The measurements were integrated by Chemstation chromatographic software interfaced to a personal computer. The analytical column was ZORBAX Eclipse XDB C18 column (15 cm x 4.6 mm I.D, 5 µm, USA). Operative conditions were: mobile phase A, 2% acetic acid; mobile phase B, acetonitrile; flow rate, 0.85 mL / min; fixed wavelength, 280 and 360 nm; injected quantity, 10 µL; elution program (%), A:B as followed: 0 min 90/10; 10 min 50/50; 15 min 20/80; 20 min 90/10; 25 min 90/10. Identification of phenolic compounds was performed by comparison with the retention times of standard substances.

b) Instrument stands at the department of Metabolomics Groups, Institute of Plant Genetics of the Polish Academy of Sciences, Poznan, Poland, with the following specifications: first instrument; ion-trap Esquire 3000 mass spectrometer equipped with ESI was operated in negative ion mode with scan range was 15-3000 m/z and scan resolution was 13000 m/z/sec [16]. The second instrument; UPLC (the Acquity system, Waters, Milford, USA) coupled to Q-Exactive hybrid MS/MS quadrupole - Orbitrap mass spectrometer (Thermo, Bremen, Germany). The system of separation Chromatography was carried out using solvent (A) water acidified with 0.1% formic acid and (B) acetonitrile (solvent B). The flow of mobile phase 0.4 mL/min was adapt to the following sequences: 0–15 min 95:5, 15–22 min 50:50, 5 min for maintained the conditions 0.2:98, then system returned to the starting conditions and was re-equilibrated for 3 min. with column C18 (150×2.1 mm, 1.7 µm). Q-Exactive MS was operated upon following settings: the HESI ion source voltage (-3kV or 3kV). The sheath gas (N_2) flow 48 L/min, auxiliary gas flow 13 L/min, ion source capillary temperature 250°C, auxiliary gas heater temperature 380°C. The CID MS/MS experiments were performed using collision energy of 15 eV. Data recording and processing were performed using the Xcalibur 4.0 software with accuracy error threshold at 5 ppm and Imported data from raw MS data to export (abf) format were packaged in MS-DIAL 4.61 an enhanced standardized untargeted lipidomics and metabolomics by using (MSP) format libraries databases [17].

2.2. Pharmacological Study.

2.2.1. *In vitro* study.

2.2.1.1. Antioxidant activity (DPPH and ABTS methods) of different MA wastes.

DPPH and ABTS free radical-scavenging activity method was adopted to measure the *in vitro* antioxidant activity of MA ethanolic, butanolic, ethylacetate, dicloromethane and petroleum ether extract; at different concentration (15.62, 31.25, 62.5, 125, 250 and 500 µg/mL) of the three parts extract; ascorbic acid and trolox was used as a positive control. The radical scavenging model for antioxidant activity, using 1,1-diphenyl-2-picrylhydrazyl (DPPH, 250mM), was performed according to Shimada *el al.* [18]. ABTS.$^+$ dissolved in water to a 7 mM concentration. ABTS stock solution with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 24 h before use Oxidation of the ABTS was performed according to Dinkova-Kostova *et al.*,[19]. The inhibition of the DPPH and ABTS radical were calculated using the following formula: % Inhibition = [(A control − A sample) / A control] ×100. Where; A is the absorbance at 517 nm in DPPH and 734 nm in ABTS.

2.2.2. *In vivo* study for Anti-Ulcerative Colitis Activity.

2.2.2.1. Materials:

2.2.2.1.1.Animals:

The present study used male Wistar albino rats, of body weights (bwt) (150-175gm) obtained from the animal house colony of the National research centre, Dokki, Giza, Egypt. The rats were kept in standard metal cages in an air conditioned room at 22 ± 3˚C, 55 ± 5% humidity and provided with standard laboratory diet and water ad libitum. The present study complies with local and national guidelines, as it was done in accordance with the guide for care and use of laboratory animals and obtained ethics committee approval certificate from National Research Centre ethics committee numbered 16/138. Experiments were performed according to the National Regulations of Animal Welfare and the Institutional Animal Ethical Committee (IAEC),and is reported in accordance with Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines.

2.2.2.1.II. Drugs and chemicals

a) Prednisolone (Sigma Chemical Co.,St Louis, MO, U.S.A.) used as reference anti-inflammatory drug and was given orally by gastric tube.
b) Acetic acid (Elgomhoreya Co, Cairo, Egypt) injected per rectum (Pr) for induction of colonic inflammation and ulceration ; a model mimicking ulcerative colitis
c) Diethyl ether (Sigma Chemical Co., St. Louis, MO, USA), for animal anaesthesia during blood sampling from retro-orbital plexus of veins and for euthanasia under anaesthesia d)Formaldehyde (Sigma Chemical Co., St. Louis, MO, USA),for fixation of postmortem tissues dissected for histopathologic examination

2.2.2.1. III. Diagnostic kits

a)Kits for determination of Liver function tests (aspartate and alanine aminotransferase) and Kidney function tests (urea and creatinine) in serum were purchased from Biodiagnostic company, Dokki, Giza, Egypt. b) Serum highly sensitive C-Reactive Protein (CRP) was measured according to the manufacturer kit using rat hs-CRP ELISA kit from Wuhan Fine Biotech. Ltd. Co., China. c) Serum Interleukin-6 was measured according to the manufacturer kit using rat IL-6 ELISA kit Cat. no. ER0042 from Wuhan Fine Biotech. Ltd. Co., China. d) Serum Anti-Neutrophil Cytoplasmic Antibodies(ANCA)was measured according to the manufacturer kit using rat ANCA ELISA kit Cat. no. SL1417Ra from SUNLONG Biotech. Ltd. Co., China.

2.2.2.2.Methods

In vivo biological studies were conducted to investigate some pharmacological activities of anti-inflammatory and colonic anti-ulcerative activities of Musa fruits, leaves and pseudo-stem extracts, after ensuring their safety.

2.2.2.2.1. Acute and Subchronic Toxicity studies:

Determination of safety of the tested herbal extracts was done by performing acute and subchronic toxicity studies

2.2.2.2.1. I. Experimental design

Acute toxicity

The extracts of *Musa* leaves, pseudo-stem and fruit peels were dissolved in distilled water then given orally (Po) in a dose of 5 g/kg to three groups of rats each consisted of five rats. A fourth group acted as negative control group and received the same volume of distilled water. The percentage of mortality was recorded 24 hours later. No mortality occurred after 24 hours. Close monitoring of animals' change in body weight, bowel habits, hair colour or behaviour, was noticed during the next two weeks [20].

Subchronic toxicity

According to the results of acute toxicity study the selected doses for chronic toxicity study were 250 and 500mg/kg

Fifty six male rats were classified equally into seven groups: Negative control group given one ml of distilled water orally (Po). Treated groups received different three parts *Musa* leaves, pseudo-stem, fruit peels extract in two doses 250,500 mg/kg, All extracts were given orally for fourteen days.

2.2.2.2.2. II. Detection of the effect of treatment on body weight

All rats in all groups were weighed before starting the experiment, after acute toxicity study and after subchronic toxicity study .Precaution was taken that the amount of daily chaw was fixed and equal for all groups.

2.2.2.2.1. III-Biochemical parameters

The animals in all groups were kept fasting for 12 hours, on the fifteenth day of the subchronic study, blood was obtained from all groups of rats after being lightly anaesthetized with ether by puncturing retro-orbital plexus, the blood was allowed to flow into a clean dry centrifuge tube and left to stand 30 minutes before centrifugation for 15 minutes at 2500 rpm to avoid haemolysis. The clear supernatant serum was separated and collected by pasteur pipette into a dry clean tube to use for determination of serum levels of Liver and Kidney function tests [21, 22].

2.2.2.2.2-Efficacy Study:

2.2.2.2.2.1. Experimental Design:

Seventy two male Wister albino rats were divided equally into 9 groups as follows: Negative control group given one ml of distilled water orally (Po). Positive control group for which colonic inflammation and ulceration were induced without previous treatment. Musa pretreated groups received leaves extract (250,500 mg/kg), pseudo-stem extract (250, 500 mg/kg) and fruits extract (250, 500 mg/kg). Prednisolone pretreated group in a dose of 2.8 µmol /kg [1]. All treatments were given orally for two successive weeks. The last dose of each treatment was administered 2 h before ulcer induction. The selected efficacy experimental dose used in the present study depended on the results of the acute toxicity and subchronic toxicity studies which had proven the safety of tested extracts.

Method of induction of colonic ulcer

All rats were fasted overnight with access to water only, before being anesthetized with ether inhalation. A polyethylene catheter (2 mm diameter) was inserted 8 cm into the lumen of the colon via the rectum (Pr). For all treated and positive control groups, an acetic acid solution (2 mL, 8%, v/v in saline) was slowly infused into the colon through the catheter. The acid solution was then aspirated and 2 mL of phosphate buffer solution (pH = 7) was infused into the rectum of each rat [23]. Negative control rats received an equi-volume saline solution devoid of acetic acid.

2.2.2.2.2.2. Biochemical parameters

Two days after ulcer induction, blood sampling and centrifugation was done [21]. The clear supernatant serum was separated and collected for determination of serum levels of systemic inflammatory marker C-reactive protein (CRP) and determination of interleukin beta six (ILβ6) and ANCA following manufacturer's instructions.

2.2.2.2.2.3. Macroscopic Examination:

All animals were sacrificed with ether and laparotomy was performed. Colonic segments (8 cm in length and 3 cm proximal to the anus) were excised, opened along the mesenteric border, washed with saline, and scored macroscopically. Gross mucosal lesions were recognized as hemorrhage or erosions with damage to the mucosal surface. The number and severity of mucosal lesions were noted and lesions were scaled as follows: Almost normal mucosa = 0, Petechial lesions = 1, one or two lesions or lesions less than 1 mm = 2, severe lesions or lesions between 1 and 2 mm = 3, very severe lesions or lesions between 2 and 4 mm = 4, Mucosa full of lesions or lesions more than 4 mm = 5. Mean ulcer score for each animal was expressed as ulcer index (U.I) and the percentage of inhibition against ulceration was determined using the expressions in the following equation [24, 25]:

UI = UN + US + UP × 10.

UI = Ulcer index, UN = Ulcer number, US = Ulcer severity, UP = Percentage of ulcerated colons

%Ulcer inhibition = 100- [U.I. in pretreated rats/ U.I. in positive control] × 100.

2.2.2.2.2.4. Histological assessment of Liver and Kidney tissue for MA extracts acute and sub-chronic toxicity studies and Colon mucosa for efficacy of the MA extracts in anti-ulcerative colitis study

Different sections from the liver, kidneys and colon were cut and fixed in 10% formalin. The tissues were then dehydrated in ethanol and embedded in paraffin bocks.The liver and kidney tissues were cut into sections of 4-µm thickness, while the colon tissues were cut into 5 µm thick sections .All tissues were stained with hematoxylin and eosin (H&E), and conventional histopathological examination was carried out under light microscopy by a pathologist who was blinded to the therapeutic strategy. Images were acquired with a Leica ICC50 HD digital camera attached to a Leica motorized light microscope system [26].

For assessment of colonic tissue damage, the tissue sections were examined blindly and the lesions were semiquantitively evaluated in ten random low power fields, as described by Amir Rashidian et al, [27]. The grading system is scaled from 0 to 5 and the details of this grading system are illustrated in Table 1 [27].

2.2.2.2.2.5. Immunohistochemistry

Immunohistochemical procedures for the demonstration of myeloperoxidase immune reactivity, a marker of neutrophil infiltrstion, in the colon were performed according to the method of Hassan NF et al. [26]. Briefly, the paraffin-embedded colon sections were deparafinized and rehydrated in ethanol. The sections were then incubated with rabbit monoclonal anti-myeloperoxidase antibody (ERP20257, Abcam). The sections were stained with diaminobenzidine (DAB) for the demonstration of the immune reaction. Finally, counterstaining with hematoxylin was carried out. MPO immunohistochemical staining was semi quantitively assessed in the colonic mucosa and submucosa in ten random high microscopic power fields, according to the % of positively stained cells as reported by Hassan NF et al. [26]. A grading system scaled from 0 to 4 was used; in which 0 = no immune staining; 1 = positive staining in ≤25% of cells in HPF; 2 = positive staining in 25%-50% of cells in HPF; 3 = positive staining in51%-70% of cells in HPF; and 4 = positive staining in ≥70% of cells in HPF [26].

2.2.2.2.2. 6. Statistical analysis

The data were expressed as means ± SE for each group. Results were analyzed using one-way analysis of variance, followed by the Tukey–Kramer test for multiple comparisons; P value of less than 0.05 was considered significant in all types of statistical tests. Graph Pad Software (Graph Pad Software Inc., La Jolla, CA, USA) (version 6) was used to carry out the statistical tests.

3. Results And Discussions

3.1. Phytochemical study.

3.1.1. Isolation and Identification of metabolites.

The crude leaf extract of MA (70% hydroalcoholic) was applied to polyamide 6 column chromatography and eluted with water methanol mixtures in the order of decreasing polarity.

All collected fraction was investigated individually by TLC chromatography.

The 20-50 % methanolic/water subfractions uploaded on polyamide 6 column using water/methanol in order of decreasing polarity. The collected subfractions was purified by Sephadex LH-20 using 50% ethanol /water as eluent to yield 5 compounds identified as, quercetin, quercetin-3-O-*β*-D-glucoside, luteolin-7-*O*-*β*-D-glucopyranoside and Gallic acid, cinnamic acid. On the other hand, the 70 % alcohol subfraction was applied to Sephadex LH-20 column chromatography and eluted with 80% ethanol/water to give one pure compound as 2-hydroxy-4-(4-methoxyphenyl)-1H-phenalen-1-one**(fig1)**.

These compound were identified by comparing their spectral data with those reported.

Compound 1 was identified as trans-cinnamic acid; it is soluble in chloroform and methanol. Moreover, **Compound 1** gives blue color under UV light at 254 nm with R_F: 0.87, ^{1}H NMR (CD3OD, 400 MHz): δ ppm = 7.63(d, J= 15.9, 1H, H-β), the remaining phenyl ring resonate at δppm 7.61 –7.57 (m, 2H) and 7.42 – 7.38 (m, 3H), 6.51 (d, J = 15.9, 1H, H-α) and ^{13}C NMR (CD_3OD, 400 MHz): δ ppm :171.65 (C=O), 145.05 (C-β), 136.16(C-1), 131.05(C-4), 129.93 (C2 & C-6), 129.00 (C-3 & C-5), 120.99 (C-α) in (Fig S2 and Table 3) [28].

Compound 2 identified as gallic acid: It soluble in methanol and gave light blue color under UV at 254 nm. ^{1}H NMR analysis (400 MHz, DMSO-d_6) δ ppm: 6.97 (S). ^{13}C NMR: (δ ppm) 165.67 (C7; C=O) 144.10 (C3& C5), 138.99 (C4), 118.82 (C1) 108.86 (C2& C6) (Fig S3 and Table 3) [29].

Compound 3 was identified as quercetin; It is soluble in methanol and give yellow color under UV light, 356 nm, ^{1}H NMR (400 MHz, DMSO): δ 7.74 (1H, d, J = 2.1 Hz, H-2′), 7.62 (1H, dd, J = 8.3, 2.1 Hz, H-6′), 6.88 (1H, d, J = 8.3 Hz, H-5′), 6.39 (1H, d, J = 2.0 Hz, H-8), 6.18 (1H, d, J = 2.0 Hz, H-6)" and ^{13}C NMR: δ (ppm) 93.2 (C-8), 98.0 (C-6), 102.9 (C-10), 115.0 (C-2'), 115.4 (C-5'), 119.8 (C-6'), 121.8 (C-1'), 135.5 (C-3), 144.9 (C3'), 146.7 (C-2), 147.5 (C-4'), 156.0 (C-9), 160.6 (C-5), 163.8 (C-7), 176.7 (C-4). Compounds 1,2 and 3 were eluted with mobile phase benzene: methanol: acetic acid,45:8:4 (Fig S4 and Table 3) [30].

Compound 4 identified as as quercetin-3-*O*-*β*- D-glucoside; it soluble in methanol gave dark purple color under UV light at 254 nm with R_F : 0.42 eluted with mobile phase butanol: acetic acid: water, 30:10:10. ^{1}H NMR (400 MHz, DMSO-d6: 7.59 (1H, d, J = 1.8, H-2), 7.62 (1H, dd, J = 1.8 & 8.4, H-6′), 6.87 (1H, dd, J =8.4, H-5′), 6.39 (1H, d, J = 2.2, H-8), 6.19 (1H, d, J = 2.4, Í-6), 5.43 (1H, d, J = 7.6,H-1″13C NMR spectrum (100 MHz, DMSO-d6, , ppm): 156.39 (C-2), 133.31 (C-3), 178.18 (C-4), 161.12 (C-5), 98.66 (C-6), 163.23 (C-7), 94.13 (C-8), 156.14 (C-9), 104.75 (C-10), 120.88 (C-1′), 117.04(C-2′), 145.25 (C-3′), 148.78 (C-4′), 114.28 (C-5′), 122.01 (C-6′), 101.09 (C-1″), 74.16 (C-2″), 76.73 (C-3″), 70.89 (C-4″), 77.65 (C-5″), 61.18 (C-6″) (Fig S5 and Table 3) [30].

Compound 5 was identified as Luteolin-7-*O*-β-D-glucopyranoside, It soluble in methanol and give dark purple under UV lamb at 254 nm with R_F: 0.891 eluted by mobile phase butanol: acetic acid: water, 30:10:10. ^{1}H-NMR (DMSO-d_6) revealed signals at *d* ppm 7.45 (*dd*, *J* = 8.3 Hz, and *J* = 2.2 Hz, H-6'), 7.42 (*d*, *J* = 2.2 Hz, H-2'), 6.9 (*d*, *J* = 8.3 Hz, H-5'), 6.8 (*d*, *J* = 2.2 Hz, H-8), 6.7 (s, H-3), 6.4 (*d*, *J* = 2.2 Hz, H-6) and signal appeared as doublet at *d* ppm 5.08 (*d*, *J* = 6.6 Hz,

H-1" of glucose) assignable for the anomeric proton of the sugar moiety and ^{13}C-NMR spectrum (DMSO-d_6). ^{13}C NMR (100MHZ, DMSO-d_6) δ (ppm): 181.92 (C-4), 164.99 (C-7), 163.14 (C-2), 161.51 (C-5), 157.97 (C-9), 149.95 (C-4'), 147.12 (C-3'), 118.88 (C-6'), 122.12 (C-1'), 116.59 (C-5'), 113.86 (C-2'), 103.89 (C-10), 103.19 (C-3), 99.82 (C-1"), 99.58 (C-6), 94.49 (C-8), 77.14 (C-5"), 76.71 (C-3"), 74.02 (C-2"), 70.11 (C-4"), 61.17 (C-6") (Fig S6 and Table 3) [31].

Compound 6 was identified as 2-hydroxy-4-(4-methoxyphenyl)-1H-phenalen-1-one, it soluble in chloroform and gave light blue under UV lamb, 254 nm. with R_F : 0.82 eluted by mobile phase methanol: chloroform, 9.5:0.5.^{1}H NMR (400 MHz, $CDCl_3$), δppm : 392(3H s, OCH_3,)7.05 (2H, d, J=8.48 Hz, H-3' and H-5'), 7.34(1H, s, H-3), 7.42 (2H, d, J= 8.48, H-2' and H-6'),7.58 (1H, d, J= 8.49, H-5), 7,81(1H, t, J=8.49 Hz, H-8), 7.95 (1H, d, J=8.49, H-6), 8.27 (1H, dd, J =1.2 & 8.3Hz, H-7), 8.78 (1H, dd, J=1.2 &8.3, H-9); ^{13}CNMR($CDCl_3$ ppm: 55.60 (OCH_3), 112.89 (C-3), 114.15 (C-3' and C-5'), 125.04 (C-9b), 126.63(C-3a), 127.77(C-8), 129.84 (C-9a), 130.14 (C-5), 131.38 (C-6), 131.65(C-6a), 131.72(C-9). 132.00 (C-2' and C-6'), 132.39 (C-1'), 136.64 (C-7), 143.99 (C-4), 149.49 (C-2), 159.75(C-4'), 179.98 (C-l) (Fig S4 and Table 3) [32].

3.1.2. GC-MS of essential oil from fruit peels.

The essential oil was extracted from fruit peels by hydrodistillation from five fours which is the suitable time to obtain the volatile oils.

Injection obtained essential oil to GC/MS resolute 37 compounds. The major compounds was isoamyl isobutyrate amounted to 18.3%, followed by Myristicine 9.31% and Isovaleric acid amounted to 8.06% of the oil. On the other hand, the oil contained n-Hexadecanoic acid amounted to 22.05%. All compounds identified are uncommon compounds found in essential oil as general. However these compounds are similar to that identified from banana reported by Heliofabia et al., [33]. These constituents of fruit peel were presented in (Table 2).

HPLC and LC-MSMS **profiles of secondary metabolites from MA.**

The metabolomics profile was identified based on low and high throughput sensitive LC/MS analyses which enabled the in-depth studies of secondary metabolite changes in MA plant with different parts as leaves, pesudostem and in fruit peels. 76 different compounds were identified including phenols, flavonoids, phenylphenalenones, amino acids and fatty acids from the agro waste of different parts of MA. LC-MSMS profile was used as a marker for the Ulcerative colitis. The individual compounds were identified via comparison of the exact molecular masses (**Δ** less than 5 ppm, mass spectra and retention times) with those of the standard compounds available in PubChem, ChEBI, Metlin, KNApSAck, HPLC, NMR and literature data. Different types of phenolic compounds of MA extracts were recorded in (Table 3) included phenolic acids and polyphenols such as gallic acid, caffeic acid, syringic acid, ferulic acid, Salicylic acid, Caffeic acid, Caffeoylquinic acid, kaempferol, catechin, Feruloylquinic acid, Vanillic acid hexoside, Sinapic acid-*O*-glucoside, and Kaempferol 3-Sophortrioside.

The biologically effects of *Musa* extract are most probably due to its content found in the different extracts. These fractions included the petroleum ether, chloroform fraction, ethyl acetate fraction, n-butanol fraction, and water fraction. In the biological activity screening tests, the n-butanol fraction showed stronger antioxidant activities than the other four fractions and it was also the potent fraction for *in vivo* efficacy study of the protective effects against ulcerative colitis**.**

Metabolomics based on high throughput sensitive UPLC-HESI-MSMS enabled in-depth studies on secondary metabolites in several parts from MA and revealed **75** different compounds, mainly phenolic, flavonoids and 12 different fatty acids. The individual compounds were identified via the exact molecular masses with Δ less than 5 ppm, mass spectra and retention times and were compared with those of the standard compounds, as well as databases available online (PubChem, ChEBI, Metlin and KNApSAck) and literature data (Table 3).

Excessive production of cytokines as Ilβ6 lead to severe inflammation which can be suppressed by natural compounds as phenolics present in natural products like *p*-coumaric acid , rutin caffeic acid which inhibits induction of lipopolysaccharide inducible nitric oxide synthase production , also flavonoids as naringenin, quercetin prevent expression of inducible nitric oxide synthase protein through inhibition of nuclear factor-κB that represents the major transcripting factor for inducible nitric oxide synthase [34].

In the current work, a comprehensive characterization of secondary metabolites using LC/MSMS was accomplished in the hydroalcoholic Musa waste extract, as well as in the oil fraction identified by GC/MS. The analysis explained 75 secondary metabolites belonging to simple phenols, amino acids, phenolic acids, cinnamic acid derivatives and flavonoids in addition to sugars. Total flavonoid and phenolic contents were more pronounced in the butanol extract. The latter also exhibited potent anti-inflammatory bowel disease “Ulcerative Colitis”

Phenolic acids are aromatic carboxylic acid with hydroxyl derivatives that have only one phenolic ring in their structure. They include two types; hydroxybenzoic acid and hydroxycinnamic acid derivatives [35]. Caffeic, p-coumaric, ferulic and sinapic acids are the hydroxycinnamic acid derivatives that are more abundant in plants as compared to the benzoic acid derivatives; such as gallic acid, protocatechuic acid and p-hydroxybenzoic acid (Table 3).

3.2. Pharmacological study.

3.2.1. In vitro study

Results of the present study revealed that the highest concentrations of IC_{50} in both DPPH and ABTS antioxidant were found in MeOH-Leaves; 5.85: 14.92, then MeOH-fruit; 9.94:12.08 and MeOH- pesudostem; 13.17:41.08, respectively) Fig 2 and Table S2. These findings are in agreement with Oresanya *et al.* [36].

3.2.2. Acute and sub chronic toxicity studies:

In the present acute toxicity study Musa leaves, pseudo-stem and fruits extracts given to three groups rats in a single dose of 5 gm/kg; all were given once; exhibited no mortalities during the first twenty four hours after administration. The percentage of body weight change of the group that received pseudo-stem extract showed significant decrease while the group that received fruit extract showed significant increase compared to negative control group (Table 4). However there weren't any changes in bowel habits, also there weren't any changes in behaviour or hair loss or discolouration in all groups during the two successive weeks duration of the experiment.

Moreover histopathologic examination of both liver and kidneys revealed normal hepatic parenchyma and normal hepatocytes **(fig 5a)**, and normal renal tubules and glomeruli **(fig 6a)**.

Accordingly the selected doses for testing the sub-chronic toxicity of all extracts were 250 and 500 mg/kg given orally for fourteen successive days, which is the same duration of the efficacy study. Observation of rats for any marked change in body weights (Table 5), or gross bowel habit changes as severe or frequent motions or severe constipation revealed that they were the same as negative control group, also their behaviour was the same as negative control group.

Assessment of both liver and kidney functions in the subchronic toxicity study by measuring ALT, AST, Urea and Creatinine levels in sera of treated rats (Table 6), in the subchronic toxicity study showed non - significant variation from negative control group.

In the present study, results of both acute and subchronic toxicity studies denoted the safety of Musa leaves, pseudostem and fruits to be used in the efficacy study as protective agents against inflammatory model of rat distal part of colon mimicking ulcerative colitis in humans.

3.2.3. Efficacy Study:

In the present study the efficacy of Musa leaves, pseudo-stem and fruit extracts was evaluated as potential protective supplements against colonic inflammatory disease in a rat model mimicking ulcerative colitis in human patients.

Ulcerative colitis was induced by per rectal injection of 2ml 8% acetic acid. Treatment with Musa extracts was given orally to rats in doses of 250 and 500 mg/kg of each extract for fourteen days prior to induction of ulcerative colitis. The doses were selected according to the results of toxicity studies formerly done in the present work.

The weights of all treated rats involved in the study were within normal and didn't show any significant difference from the negative control group, also the % change of weights at the end of experiment compared to those before starting was minimal. The non significant change in body weights denotes that the extracts don't alter normal bowel habits and don't affect the appetite of rats as food consumption was constant throughout the experiment (Table 5).

It was noticed that untreated positive control rats suffered severe diarrhoea within the twenty four hours period following acetic acid per rectal infusion for induction of UC. This finding varied in intensity from mild to absent in all other groups, which denotes that acetic acid led to severe irritation.

Evaluation of the effects of pretreatments was performed by macroscopic examination of dissected colons by naked eyes (table 8), and by histopathologic examination (table 7) followed by immune-histochemical examination (table 9), and finally biochemical assay for detection of inflammatory markers (table 10), in addition to the qualitative test antineutrophil cytoplasmic antibodies(ANCA) which is specific for UC detection.

Macroscopic examination of colons dissected from negative control group showed intact mucosa with no signs of inflammation or haemorrhagic spots (score 0). Microscopic examination of mucosa of colons of rats in this group was normal and the lamina propria was normal with few eosinophils and normal crypts that were lined by mucin-secreting cells **(fig 3a&b)** , and both submucosa and T-muscularis**(fig 4a&b)**were also normal which was consistent with gross examination of negative control colons.

On the other hand colons dissected from untreated rats that received only acetic acid per rectum were severely ulcerated to the degree of perforation with grossly detected haemorrhagic areas in 100% of rats, which was also confirmed by histopathologic examination as severe deleterious histopathological lesions were demonstrated in the colon of Positive Control (C+ve) group, with increased pathologic lesion scoring. These histopathological lesions were characterized by diffuse ulcerative colitis with diffuse necrosis and desquamation of mucosal epithelium and complete necrosis as well as fragmentation of the crypts which are intensely infiltrated by neutrophils in addition to severe congestion of mucosal blood vessels **(fig 3c)** in addition to aggregation of bacterial colonies **(fig 3d)**. The submucosa and tunica muscularis are greatly expanded by edematous fluids and neutrophilic cell infiltration **(fig 4c & 4d**, respectively). Liver showed mild granular degeneration of hepatocytes **(fig 5b)**. Vacuolation of individual cells lining the renal tubules were demonstrated in the kidneys of this group **(fig 6b).**

On the other hand macroscopic examination of group treated with prednisolone which was used as a standandard drug revealed significant reduction in ulcer index as 62.5% of rats were affected and showed significant increase in percentage of ability of protection against UC (41.81%) compared to untreated group,the results were consistent with histopathology , which revealed pronounced improvement with significant decrease of pathologic lesion scoring, which revealed small multifocal ulcerative lesions with focal necrosis and desquamation of mucosal epithelium, focal mononuclear inflammatory cell infiltration **(fig 3e)** and few proprial hemorrhage **(fig 3f).** The submucosa and T.muscularis are infiltrated by few neutrophils **(fig 4e & 4f**, respectively). Mild focal vacuolar degeneration of hepatocytes was demonstrated in the liver **(fig 5c)**, but normal renal tubules were demonstrated in the kidneys **(fig 6c)**. .

In contrast to Prednisolone, gross examination by naked eye of group treated with leaves 250mg/kg revealed increased number and severity of ulcers in 87.5% of pretreated rats, with no significant improvement where the ability of protection against ulceration was only 15.1%, and that was confirmed by histopathology as there was diffuse necrosis of colonic mucosa associated with severe congestion of mucosal blood vessels and massive neutrophilic cell infiltration were frequently observed **(fig 3g & 3h)**.In addition, intense infiltration of the submucosa with neutrophils was marked (fig 4g). The T.muscularis

revealed marked separation of muscle fibers by edematous fluid and leukocytic cell infiltration **(fig 4h).** Swelling and vacuolation of hepatocellular cytoplasm were demonstrated in the liver **(fig 5d).** In addition, vacuolization of some renal tubular epithelial cells were demonstrated in the kidneys **(fig 6d).**

In comparison to low dose leave group, significant amelioration was recorded in the high dose leave group (500 mg/kg), by gross examination as the ulcer index was significantly reduced and the percent of protection of the high dose extract was 28.31% which was significantly higher than both low leave extract and positive control group as ulceration was detected only in 75% of pretreated rats, yet it was significantly less than prednisolone group. Consistently histopathologic examination revealed large focal erosive lesion and few proprial hemorrhage **(fig 3i & 3j).** But the sub mucosa and T.muscularis were intensely infiltrated with neutrophils **(fig 4i & 4j**, respectively). The liver and kidneys of this group appeared normal **(fig 5e & 6e**, respectively).

The group pretreated with pseudostem 250 mg/kg showed better macroscopic examination profile as the number and severity of ulcers was less as they were detected in only 75% of pretreated rats which consequently significantly reduced the ulcer index compared to leaves pretreated group by low and dose and also to positive control group, but its protective effect was significantly less than prednisolone and almost the same as leaves pretreated group with high dose as the % of protection was 28.52 % in pseudostem low dose group .The histopathologic examination of pseudostem low dose revealed pronounced attenuation of the pathological lesions with decreases pathologic lesion scoring,small focal necrosis of mucosal epithelium associated with mild proprial edema and few leukocytic cell infiltration were demonstrated **(fig 3k & 3l)**. Mild infiltration of submucosa and T.muscularis with neutrophils was recorded in this group **(fig 4k & 4l**, respectively). Normal histological structures of liver and kidneys were also demonstrated **(fig 5f & 6f**, respectively).

Regarding the gross examination of the high dose of pseudostem (500 mg/kg) and low dose of fruit extract (250 mg/kg), ulcers were detected in only 62.5% ,which is the same percentage of affected rats in the prednisolone (standard),also the number and severity of ulcers were approximately close to each other leading to non significant differences in ulcer indices and consequently % of protection of both pseudostem extract high dose (40.34%) and fruit extract low dose (41.13%)on one side, and prednisolone (41.81%) which is the standard treatment on the other side, however their protective effects were significantly higher than those of leaves low and high doses as well as pseudostem low dose, and of course the positive control group. On the other hand they showed significant lower protective effects than fruits extract high dose (500 mg/kg), whose protective effect was the highest of all pretreatments when compared to positive group and each pretreatment with pronounced significant % of protection of 53.33% and least number of ulcers, severity and affection of only 50% of rats, consequently exhibiting the least ulcer index among all other groups. The histopathologic photomicrographic findings were consistent with the macroscopic examination of these groups, where normal colonic mucosa in most examined sections was frequently demonstrated in high dose stem-treated groups stem group. Regenerative activity of the mucosal epithelium and minimal leukocytic cell infiltration as well as scant proprial hemorrhage were demonstrated in high dose stem-treated groups **(fig 3m & 3n).**The submucosa was infiltrated with few neutrophils **(fig 4m)** and the T.muscuaris showed edema with few neutrophilic cell infiltration **(fig 4n)**. Normal hepatocytes and renal parenchymal structures were also demonstrated **(fig 5g & 6g**, respectively). Much better improvement, with marked regenerative activity of the colon mucosa and proliferation of colonic lymphoid nodules and minimal leukocytic cell infiltration were recorded in the mucosa of low dose fruit treated groups **(fig 3o & 3p)** and high dose fruit treated groups **(fig 3q & 3r).** The submucosa and T.muscularis of low dose fruit treated groups were mildly infiltrated with neutrophils **(fig 4o & 4p)**. Only mild focal congestion of some hepatic sinusoids were demonstrated in this group **(fig 5h)**, but normal histological structures were demonstrated in the kidneys **(fig 6h)** Sparse neutrophils were demonstrated in the submucosa and T.muscularis of high dose fruit treated groups **(fig 4q & 4r)**. Normal heptic and renal parenchyma were demonstrated **(fig 5i & 6i,** respectively).

The results of *MPO immune-histochemical expression* recorded in the colonic mucosa and submucosa showed that individual MPO+ cells were demonstrated in the mucosa and submucosa of the colon of normal rats **(fig 7a & 8a)**. Whereas, increased expression of MPO with significant increase of % of MPO+ cells was recorded in the colonic mucosa and submucosa of C+ve group **(fig 7b & 8b)**. Significant decrease of % of MPO+ cells was recorded in the mucosa and submucosa of Prednisolone-treated group **(fig 7c & 8c)**. The colon of low dose leave group showed increased % of MPO+ cells, which are insignificantly different from the C+ve group, in the mucosa and submucosa **(fig 7d & 8d)**. But significant difference was recorded in the high dose leave group in both the mucosa and submucosa **(fig 7e & 8e)**. Better improvement with marked decrease of % of MPO+ cells was recorded in the mucosa **(fig 7f & 7g)** and submucosa **(fig 8f & 8g)** of low and high dose stem-treated groups, with insignificant difference between them. On the other hand, significant difference was recorded between low and high dose fruit treated groups. Remarkable decrease of MPO expression with significant decrease of MPO+ was recorded in the mucosa and submucosa of low dose fruit treated groups **(fig 7h)**. Only few scattered MPO+ cells were demonstrated in the mucosa and submucosa of high dose fruit treated groups **(fig 7i & 8i)**, while low dose fruit treated group showed significant decrease of MPO+ cells with brown staining **(fig 8h)**.

Biochemical analysis of sera obtained from rats infused per rectally with acetic acid in this study aiming at inducing a rat model of UC ,revealed highly significant elevation of inflammatory markers CRP and Ilβ6 in the untreated group positive control compared negative control and to all other treated groups. The degree of inflammation was variable between the treated groups, all showed significant protection but the highest were those of the groups given prednisolone and fruit extract in high dose as these groups showed non significant difference from the negative control group.

Regarding ANCA test which is a highly specific qualitative test for diagnosis of UC, it revealed 100% negativity in the negative control group and in all treated groups except leaves low dose where it was 75% negative. On the contrary, the positive control results were 100% positive. This finding is in enforced by Pang et al, [37] in their study as they stated that ANCA test is diagnostic for UC and its quantification reveals the severity of UC[37].

The anti-inflammatory effects of phenolics present in Musa extract when they were given orally in our study are due to serial enzymatic reactions that take place in the digestive system. After being absorbed in the small intestine they conjugate with glucuronic acid and sulfonate, then 5%-10% pass to the plasma [38], but the largest portion (90–95%) pass directly to the large intestine (colon) [39]. where fermentation occurs by colonic microbiota ,leading to elaboration of the positive effect of phenolics on colon's health by reducing its pH as anti-inflammatory effects [38],which was emphasized in our study and consequently suppression of cancer cells.

Conclusion

In the present study an animal model mimicking Ulcerative colitis was induced by using per rectal acetic acid infusion, it produced severe inflammation which could be prohibited by pretreatment with natural plant extracts rich in Flavonoids and phenolic acids due to their protective effects on the colon as 90-95% of phenolic acids are metabolized in the colon by microorganisms that lead to their anti-inflammatory activity.

This was clear in our study as characterization of secondary metabolites in Musa revealed high contents of these compounds.

It is noteworthy mentioning that the Musa fruits had the best effect regarding the ability to protect against development of severe Ulcerative colitis ,which introduces a promising natural supplement that can be used in future clinical studies for further evaluation of its effect as a protective pretreatment in vulnerable patients that are susceptible to have Ulcerative colitis.

Declarations

Conflicts of interest:

The Authors have no conflicts of interest to declare.

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Tables

Table 1. Grading system of colonic ulcers.

Grades	Histopathological criteria
Grade 0	Normal histological structure of colonic mucosa, submucosa and T.muscularis with no inflammatory cell infiltration
Grade1	Infiltration of the colonic mucosa and submucosa with Inflammatory cells
Grade 2	Infiltration of the colonic mucosa, submucosa and T.muscularis (trans-mural) with Inflammatory cells
Grade 3	Focal ulceration with trans-mural inflammation
Grade 4	Multiple large ulcers with trans-mural inflammation
Grade 5	Extensive ulcerations with complete necrosis of the colonic mucosa and intense trans-mural inflammation

Table 2. The main constituents of the essential oil peel waste fruit *Musa acuminata*.

No	Name compounds	Formula	Rt	Area Sum %
1	3-methyl butanol	$C_5H_{12}O$	3.15	0.89
2	Isobutyl acetate	$C_6H_{12}O_2$	3.81	0.34
3	2-Pentanol, acetate	$C_7H_{14}O_2$	5.51	1.02
4	1-Butanol, 3-methyl-, acetate	$C_7H_{14}O_2$	6.24	2.99
5	Butanoic acid, 2-methylpropyl ester	$C_8H_{16}O_2$	8.70	2.46
6	Butanoic acid, butyl ester	$C_8H_{16}O_2$	10.08	1.42
7	Isobutyl isovalerate	$C_9H_{18}O_2$	10.43	0.51
8	Butanoic acid, 1-methylbutyl ester	$C_9H_{18}O_2$	11.11	3.77
9	2-Heptanol, acetate	$C_9H_{18}O_2$	11.74	2.55
10	Butanoic acid, 3-methyl-, butyl ester	$C_9H_{18}O_2$	11.86	0.50
11	**isoamyl isobutyrate**	**$C_9H_{18}O_2$**	**12.25**	**18.3**
12	**Isovaleric acid**	**$C_{10}H_{20}O_2$**	**13.99**	**8.06**
13	1,3,8-p-Menthatriene	$C_{10}H_{14}$	14.13	0.49
14	Butanoic acid, hexyl ester	$C_{10}H_{20}O_2$	17.07	1.09
15	Butanoic acid, 1-cyclopentylethyl ester	$C_{11}H_{20}O_2$	17.87	3.56
16	Butanoic acid, 3-methyl-, hexyl ester	$C_{11}H_{22}O_2$	18.78	1.22
17	Isopentyl hexanoate	$C_{11}H_{22}O_2$	19.06	0.75
18	Butanoic acid, 1-ethenylhexyl ester	$C_{12}H_{22}O_2$	23.26	0.48
19	Hexanoic acid, undec-10-enyl ester	$C_{17}H_{32}O_2$	23.59	0.25
20	Z,Z,Z-4,6,9-Nonadecatriene	$C_{19}H_{34}$	24.60	0.39
21	Cyclohexane, ethylidene-	C_8H_{14}	24.81	0.93
22	(Z)-5-Octen-1-ol	$C_8H_{16}O$	25.15	0.35
23	cis-.beta.-Farnesene	$C_{15}H_{24}$	25.77	0.24
24	β-Cubebene	$C_{15}H_{24}$	26.49	0.45
25	**Myristicine**	**$C_{11}H_{12}O_3$**	**27.77**	**9.31**
26	Benzene, 1,2,3-trimethoxy-5-(2-propenyl)-	$C_{12}H_{16}O_3$	28.81	1.39
27	Bicyclo[8.2.0]dodecane, 11,11-dimethyl-	$C_{14}H_{26}$	29.03	0.23
28	Cyclohexane, 1-methylene-3-(1-methylethenyl)-, (R)-	$C_{10}H_{16}$	30.86	0.4
29	Apiol	$C_{12}H_{14}O_4$	31.11	0.29
30	1,1'-Biphenyl, 3,4-diethyl-	$C_{16}H_{18}$	33.30	0.33
31	Phthalic acid, butyl undecyl ester	$C_{23}H_{36}O_4$	41.30	0.85
32	Palmitoleic acid	$C_{16}H_{30}O_2$	43.86	0.6
33	**n-Hexadecanoic acid**	**$C_{16}H_{32}O_2$**	**44.76**	**22.65**
34	11-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	48.09	0.56
35	Phytol	$C_{20}H_{40}O$	48.60	1.65
36	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	49.04	4.21
37	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{18}H_{30}O_2$	49.20	4.57

Table 3. HPLC and LC-MSMS data in low and high resolution of different MA parts identified by tentative mass.

No	Tentative Identification	RT	Chemical formula	Low mass	High resolution Mass			UV λ_{max}	*Musa acuminata*		
				[M-H]-	measured &caculated	mass of [M-H]-	Δ ppm		1	2	3
Phenolic acids and Phenolic glycosides											
1	Vanillin	1.63	$C_8H_8O_3$		151.0397, 151.0390	136.0152, 108.0202, 93.0332, 69.0702	-1.7152	216, 259	*878.06	*23.17	-
2	L-Tyrosine methyl ester	2.04	$C_{10}H_{13}NO_3$	194.25, 176.24, 128.23, 84.43	194.0812, 194.0812	176.0706, 162.0561, 118.1188, 96.0562	0.0361	206, 263	*	*	-
3	Bergapten[a]	2.12	$C_{12}H_8O_4$		215.0320, 215.0339	179.0555, 131.0447, 113.0228, 89.0227, 71.0121	7.4296	206,224	*	-	*
4	Quinic acid[a]	2.15	$C_7H_{12}O_6$		191.0551, 191.0550	173.0444, 127.0386, 111.0072, 85.0279, 59.0122	0.6044		*	-	*
5	Threonic acid[d]	2.14	$C_4H_8O_5$		135.0285, 135.0293	117.0179, 89.0227, 75.0071	-6.3694	202, 220	*	-	
6	Caffeoyl quinic acid[a]	2.17	$C_{16}H_{18}O_9$		353.0853, 353.0867	263.0588, 173.0443, 155.0337, 113.0228, 89.0227	-3.9987	202	*	-	*
7	N-Fructosyl isoleucine[d]	2.18	$C_{12}H_{23}NO_7$		292.1401, 292.1403	227.0654, 203.0667, 173.0555, 141.0655, 130.0859	3.497	220	*	-	*
8	N-Fructosyl pyroglutamate[d]	2.40	$C_{11}H_{17}NO_8$		290.0883, 290.0884	254.0660, 212.0555, 200.0555, 170.0447, 128.0338	-0.2154	216	*	-	*
9	Tyrosine[d]	2.52	$C_9H_{11}NO_3$		180.0656, 180.0655	163.0389, 119.087, 93.0330	0.6865	262, 357	*	-	*
10	Methoxytyrosine[d]	3.64	$C_{10}H_{13}NO_4$		210.0765, 210.0761	128.0389, 118.0647, 94.0282, 66.0332	2.1598	221.335	*	*	*
11	Acetylleucine[d]	3.86	$C_8H_{15}NO_3$		172.0965, 172.0968	130.0859, 59.0803	-1.6122	209, 259, 335	*	*	*
12	Tryptophan[d]	3.88	$C_{11}H_{12}O_2N_2$		203.0818, 203.0815	142.0652, 116.0489, 97.0283	1.2432	215, 239, 258	*	*	-
13	Gallic acid[b,c]	2.66	$C_7H_6O_5$		169.0132, 196.0131	125.0229, 97.0281, 65.0016	0.2002	258. 288, 373	* 10.01	-	* 20.01
14	Salicylic acid[d]	2.93	$C_7H_6O_3$		137.0232, 137.0239	93.0330	-4.7078	220	*	*	*
15	Benzoic acid[d]	3.56	$C_{13}H_{16}O_9$		315.0717, 315.0711	165.0176, 152.0101, 132.0005, 108.0200, 85.0276	2.0880	202, 252	*	*	-
16	Cinnamyl alcohols[a]	3.78	$C_9H_{10}O_3$		165.0546, 165.0546	147.0439, 119.0487, 72.9915	-0.0573	262, 319, 380	*	-	*
17	Caffeoylquinic acid[a]	3.91	$C_{16}H_{18}O_9$		353.0876, 353.0880	191.0562, 180.0377, 161.0228, 135.0435	2.525	254, 348	*	*	*
18	Caffeic acid[b,a]	4.00	$C_9H_8O_4$		179.0342, 179.0339	135.0437, 103.9188,	1.9857	241, 335	-	*25.46	*106.27

						71.0122					
19	P-Coumaric acid[b]	4.09	$C_9H_8O_3$		163.0389, 163.0395	119.0488	-4.0167	245	-	* 162.91	* 17.16
20	Ferulic acid[b,c]	4.11	$C_{10}H_{10}O_4$		193.0492, 193.0495	178.0259, 149.0599, 134.0357	1.6080	241, 331	* 29.76	-	* 59.60
21	Citric acid[a]	4.22/ 3.2	$C_6H_8O_7$	190.20, 110.26	191.0184, 191.0186	111.0071, 87.0070	-1.4275	241, 320	*	-	-
22	Propyl gallate[b]	4.25	$C_{10}H_{12}O_5$		211.0242, 211.0237	167.0339, 123.0437, 93.0330, 65.0381	2.1586	-	* F4713.85	* 34.70	* 20.50
23	Vanillic acid hexoside[a]	4.91	$C_{14}H_{18}O_9$		329.0884, 329.0867	195.1921, 167.0340, 109.0281	5.0758	202, 209	*	*	*
24	Sinapic acid-*O*-glucoside[a]	4.97	$C_{17}H_{22}O_{10}$		385.1148, 385.1166	263.0767, 223.0605, 205.1228, 173.0441, 153.0909	4.6739	241, 335	*		*
25	Cinnamic acid[b]	5.39	$C_9H_8O_2$		147.0444, 147.0441	-	2.2565	256, 356	*45.91	*61.32	*83.63
26	4-*O*-Feruloylquinic acid[a]	5.58	$C_{17}H_{20}O_9$		367.1024, 367.1024	193.0498, 173.0443, 134.0358, 93.0327	0.0014	263, 349	*	*	
27	Kaempferol 3-Sophortrioside[a]	5.86/ 15.4	$C_{33}H_{40}O_{21}$	771.17, 609.08, 300.44, 178.20	771.2281, 771.2289	609.1613, 301.0349	1.0373	241, 263	*		*
28	Quercetin 3-*O*-glucosyl - glucoside[a]	5.90 /20.5	$C_{30}H_{26}O_{15}$	625.12, 462.90, 300.50	625.1530, 625.1550	463.0885, 301.0351	3.1992	241,335	*		
29	Diosmetin-7-*O*-rutinoside[a]	6.31/ 21.0	$C_{28}H_{32}O_{15}$	607, 299.46, 270.41	607.1668, 607.1685	299.0199, 270.0160	2.7998	241, 267, 310	*	*	*
30	Kaempferol-3-*O*-rutinoside[a]	6.35/ 23.09	$C_{27}H_{30}O_{15}$	593.09, 284.45, 254.35, 189.16	593.1531, 593.1501	485.1238, 388.0862, 285.0401	5.0235	241, 270, 310	*		
31	Peonidin 3-rutinoside[a]	6.39	$C_{28}H_{33}O_{15}$	608.12, 341 63	608.1401, 608.1470	343.0461, 300.0273, 271.0236, 178.9975, 125.0230	11.3460	241, 270, 310	*	*	
32	Sulfo jasmonate[d]	6.41	$C_{12}H_{18}O_7S$		305.0699, 305.0690	255.1124, 96.9585	2.9656	241, 306, 349		*	*
33	Luteolin-7-O-β-D-glucopyranoside[b,d]	6.48	$C_{21}H_{20}O_{11}$		447.0929, 447.0922	285.0405	1.5147	241, 320	* 210.21	* 50.23	* 120.56
34	Delphinidin 3-rutinoside[a]	6.53	$C_{27}H_{31}O_{16}$		610.1607, 610.1603	301.0324, 272.0288, 151.0024	-0.6555	241,270, 306, 320	*	-	-
35	Quercetin *O*-rhamnoside-*O*-hexoside[a,b]	6.56/ 22.2	$C_{27}H_{30}O_{16}$	609. 11, 300.48, 242.59	609.1554, 609.1560	343.0462, 301.0716, 242.0568, 151.0023	0.9849	241, 270	*	-	*
36	Naringin[b]	6.55/ 23.4	$C_{27}H_{32}O_{14}$	579.13, 458.95, 270.45	579.1782, 579.1771	459.1146, 402.1311, 339.0713, 271.0612, 235.0239, 181.0490, 151.0023	-1.8992	241, 320	*470.93	*19.16	-
37	Quercetin-3-O-glucoside[c]	6.65/ 24.0	$C_{21}H_{20}O_{12}$	462.96, 300.46	463.0886, 463.0871	300.0273, 271.0241, 151.2179	3.2179	241, 277, 320	*	*	*

38	Quercetin-3-O-rutinoside[a,b]	6.70/ 22.8	$C_{27}H_{30}O_{16}$	609.08, 300.44	609.1576, 609.1580	325.700, 301.0707, 242.0578, 151.0020	0.6566	241, 277, 320	*3806.15	*480.14	*50.08
39	isorhamnetin-3-O-galactoside-6''-rhamnoside[a]	6.76/ 24.1	$C_{28}H_{32}O_{16}$	623.12, 314.56, 466.90	623.1368, 623.1370	463.0908, 357.0613, 314.0431, 271.0251, 151.0024	0.3209	241, 270, 320	*	*	-
40	Kaempferol-3-O-glucoside[a]	6.79/ 24.9	$C_{21}H_{20}O_{11}$	446.97, 283.42, 254.42, 150.15	447.0931, 447.0922	284.0325, 255.0300, 151.0031	2.0608	241, 320	*	*	*
41	Apigenin-7-O-neohesperidoside[a]	6.94/ 25.0	$C_{27}H_{30}O_{14}$	577.87	577.1615, 577.1617	460.0598, 269.0454, 175.0395	0.3465	241, 270, 306	*	-	*
42	isorhamnetin-3-O-glucoside[a]	7.08/ 25.2	$C_{22}H_{22}O_{12}$	476.96, 356.58, 313.61, 242.32	477.1044, 477.1028	357.0601, 314.0432, 243.0270, 258.0270, 151.0027	3.4546	241, 270, 320	*	-	*
43	Dodecyl sulfate[a]	7.33/ 26.3	$C_{12}H_{26}O_4S$	264.42	265.1479, 265.1468	219.8447, 185.1164,96.9584	3.9646	241, 270, 306	*	-	-
45	2-Methoxycinnamic acid[d]	7.67	$C_{10}H_{10}O_3$	178.16	177.0545, 177.0546	162.0311, 145.0279, 121.0277	-0.7429	241, 306	*	-	*
46	Daidzein[b]	7.64	$C_{15}H_{10}O_4$		253.1444, 253.1434	191.14440, 125.0956	3.6650	241, 267	-	*14.22	-
47	Aloe-emodin[d]	7.76	$C_{15}H_{10}O_5$		269.0456, 269.0444	225.1853	4.1364	246, 275	*	-	-
48	Catechin[b,c]	8.12	$C_{15}H_{14}O_6$		289.0507, 289.0495	245.0601, 217.0650	3.9939	243, 281	*908.23	-	-
49	Delphinidin[d] 7-hydroxyflavonoids	8.20	$C_{15}H_{11}O_7$		301.0354, 301.0343	273.0391, 178.9977, 151.0023, 121.0278	2.8125	241, 277, 320	-	*	*
50	Quercetin[b,c]	8.35	$C_{15}H_{10}O_7$		301.0356, 301.0343	178.9977, 151.0025, 121.0277, 83.0123	3.8263	245	* 1222.64	* 379.66	* 183.59
51	Atractylenolide III[d] (Sesquiterpenoid)	8.80	$C_{15}H_{20}O_3$		247.1336, 247.1329	203.1433, 185.1327, 169.1007	3.0975	241, 306	*	-	*
52	Wogonin[d] (8-O-methylated flavonoids)	11.15	$C_{16}H_{12}O_5$		283.0611, 283.0601	268.0374, 157.0088	3.5196	-	*	-	*
53	Kaempferol-4'-methyl ether[d] (Flavonols)	9.00	$C_{16}H_{12}O_6$		299.0562, 299.0550	284.0326, 256.0372	4.1108	241, 310, 403	*	-	*
54	Kaempferol[a]	9.13	$C_{15}H_{10}O_6$		285.0405, 285.0394	151.0033	4.0797	245, 310	-	*	*
Phenylphenalenones											
56	2-(4′-hydroxyphenyl)-1,8-naphthalic anhydride	9.20	$C_{18}H_{10}O_4$		289.0507, 289.0495	245.0610, 221.1175, 176.1829	3.9939	245, 270	*		
57	Musanolone E	9.23	$C_{19}H_{12}O_4$		303.0662, 303.0652	285.0555, 259.0756	3.3237	245, 270	*		
58	2,3-Dihydro-4-(4-methoxyphenyl)-1H-	9.50	$C_{20}H_{18}O_4$		321.1129, 321.1121	306.0895, 212.0462,	2.3325	245, 277	*		

	phenalene-1,2,3-triol				199.1693					
59	**2,3-dihydro-2,3-dihydroxt-4-(4′-hydroxyphenyl) phenalen-l-one**	9.76	$C_{19}H_{14}O_3$	305.0813, 305.0808	277.0876, 249.0900, 180.0665, 108.0201	1.6191	245, 277	*	-	*
60	**2-hydroxy-4-(4-methoxyphenyl)-1H-phenalen-1-one**	9.93	$C_{20}H_{14}O_3$	301.0861, 301.0859	286.0634, 258.0685, 176.1632	0.6636	245, 277	*	-	-
61	**Irenolone**	9.95	$C_{19}H_{12}O_3$	287.0713, 287.0703	259.0766, 107.0167	3.6536	245, 270	*	-	-
62	**Anigorufone**	10.13	$C_{19}H_{12}O_3$	287.0713, 287.0703	259.0762, 138.1300	3.4410	-	*		*
63	**Hydroxyanigorufone**	10.96	$C_{19}H_{12}O_2$	271.1807, 271.1798	253.1807, 209.1911	3.5101	-	*		*
Fatty acid, triterpenes and Lipids										
64	**Isopropylmalic acid**[d] **|Hydroxy fatty acids**[d]	3.77	$C_7H_{12}O_5$	175.0600, 175.0601	131.0697, 115.0385, 85.0642	-0.4976	241, 306	*	-	
65	**Azelaic acid**[d]	5.44	$C_9H_{16}O_4$	187.0966, 187.0965	125.0958, 111.0072, 97.0643	0.5488	242, 326	*		
66	**Hydroxysebacic acid**[d]	5.97	$C_{10}H_{18}O_5$	217.1074, 217.1071	171.1016, 155.1063, 88.1209	1.5718	241, 335	*		
67	**FA 18:2+40**[d] Long-chain fatty acids	7.48	$C_{18}H_{32}O_6$	343.2124, 343.2115	292.9891, 211.1328, 59.0123	2.6739	241, 277, 306	*		
68	**A 18:2+3O**[d] |Lineolic acids and derivatives	8.49	$C_{18}H_{32}O_5$	327.2176, 327.2166	309.2063, 291.1964, 229.1443, 211.1334, 181.1380, 171.1015, 137.0952, 113.0243, 85.0279	3.0249	241, 277, 306	*	*	*
69	**FA 18:1+3O**[d] |Long-chain fatty acids	8.52	$C_{18}H_{34}O_5$	329.2332, 329.2323	314.0430, 259.1550, 229.1433, 171.1011, 101.0676	2.8376	241, 277, 306	*		
70	**FA 18:1+2O**[d]	8.88	$C_{18}H_{34}O_4$	313.2385, 313.2373	285.0403, 178.9978, 155.1066	3.6997	249, 275	*	*	*
71	**FA 18:4+2O**[d] |Lineolic acids and derivatives	8.95	$C_{18}H_{30}O_4$	309.2053, 309.2060	291.1965, 221.1532, 209.1177, 195.1019, 171.1018, 113.0950	-2.5053	249, 272	*	*	*
72	**FA 18:4+2O**[d] |Lineolic acids and derivatives	9.46	$C_{18}H_{28}O_4$	307.19186, 307.18979	289.1808, 259.1724, 235.1337, 211.1334, 185.1170, 121.0642	2.07	245, 349	*		*
73	**FA 18:1+1O**[d] **Lineolic acids**	9.97	$C_{18}H_{34}O_3$	297.2434, 297.2424	297.2329, 253.2476, 183.0114, 155.1065, 127.1114	3.2172	272	*		*
74	**FA 18:3+1O**[d]	11.11	$C_{18}H_{30}O_3$	292.69, 274.55, 223.39, 194.36	293.2123, 293.2111	275.2015, 223.1699, 211.1334, 183.1018, 171.1016,	3.9528	249	*	*	*

					155.1068, 121.1008					
75	**LPE 16:0**[d]	11.73	$C_{21}H_{44}NO_7P$	452.2786, 452.2785	294.9081, 255.2330, 234.1745, 92.9932		-	*	*	*
76	**Corosolic acid**[d] (Triterpenoids)	12.39	$C_{30}H_{48}O_4$	471.3472, 471.3469	354.3522	0.6621	-	-	*	*

Compounds listed in the Table were found in (1) total extract of **leaves MA**, (2) Pesudostem , and (3) Peel Fruit, (a) compounds compared with literature [7, 40-43], (b) compounds identified from Standard Authentic compound Quantitative with HPLC (conc. as µg/g), (c) compounds identified from NMR , (d) compounds identified from DataBase (MS-Dial, **KNApSAcK**, Metlin, and **RIKEN).**

Table 4. Body weight changes during the Acute toxicity study of Musa leaves , pseudostem and fruits extracts**.**

Group		Negative control	Musa leaves (5000mg/kg)	Musa pseudostem (5000mg/kg)	Musa fruits (5000mg/kg)
Onset of Body weight measurement	Baseline body weight (gm)	160.6 ± 1.07	160.66 ± 1.07	160.66 ± 1.07	160.66 ± 1.07
	Body weight (gm)After two weeks	172.36 ± 5.8	1706 ± 3.7	177.86 ± 4.6	1666 ± 6.3
% of body weight increase after 2 weeks		7.286 ± 0.16	5.856 ± 0.43*	3.366 ± 0.2@*#	10.76 ± 0.57@

Results are expressed as means of body weights of rats (gm)± SE after fourteen days of single oral dose of Musa. n = 5; Data were analysed using one way analysis of variance (ANOVA) followed by Tukey Kramer's multiple. comparison test; Significance was considered at $P \leq 0.05$.

@Significantly different from negative control group, *Significantly different from Musa fruits group,# Significantly different from Musa leaves group

Table 5. Body weight changes during the Sub chronic toxicity and Efficacy study of Musa leaves ,pseudostem and fruits extracts

Onset Groups	Baseline body weight (gm)	Body weight (gm)After Two Successive weeks
Negative control	155.8±2.8	163.3±3.33
Musa leaves (250 mg/kg)		169±5.5
Musa leaves(500 mg/kg)		166.3±4.48
Musa pseudostem(250 mg/kg)		156.7±4.41
Musa pseudostem(500 mg/kg)		150.7±3.84
Musa fruits(250 mg/kg)		162.7±1.3
Musa fruits(500 mg/kg)		167.7±1.45

Results are expressed as means of body weights of rats in gm ± SE. n = 8; Data were analysed using one way analysis of variance (ANOVA) followed by Tukey Kramer's multiple comparison test. No significant difference detected among groups.

Table 6. Effect of oral administration of Musa leaves ,pseudostem and fruits extracts on Liver and Kidney Function tests of rats in subchronic toxicity study.

Parameter Groups	ALT(U/L)	AST(U/L)	Urea(mg/dl)	Creatinine(mg/dl)
Negative control	36.08±2.54	27.78±1.14	30.1±1.74	0.95± 0.03
Musa leaves (250 mg/kg)	34.9±0.38	21.22±0.9	27.52± 2.9	0.84±0.05
Musa leaves (500 mg/kg)	30.05±0.31	24.32±1.8	24.5± 3.84	0.79± 0.04
Musa pseudostem (250 mg/kg)	40.97±2.29	27.64±2.96	36.49± 0.07	0.93 ±0.03
Musa pseudostem (500 mg/kg)	35.43±3.29	22.51±2.33	27.52± 0.78	0.71± 0.05
Musa fruits (250 mg/kg)	41.9±1.64	23.17±1.7	33.15± 0.54	0.72±0.1
Musa fruits (500 mg/kg)	27.92±0.72	26.78±2.8	22.14± 1.87	0.78 ±0.02

Results are expressed as means of levels of ALT, AST, Urea and Creatinine in rat sera ± SE. n = 8; Data were analysed using one way analysis of variance (ANOVA) followed by Tukey Kramer's multiple comparison test. No significant difference detected among groups.

Table 7. Macroscopic grading of the protective effect of oral administration of Musa leaves, pseudostem and fruits extracts on Colonic Ulceration.

Ulcer Grading Groups	Number	Severity	Ulcer Index	% of Protection
Negative control	----------	----------	----------	----------
Positive Control Acetic acid 8% (2ml/rat)	5	5	1100	----------
Prednisolone (2.8 µmol /kg)	1	0.5	640±4.2$^{@}$	41.81±0.38
Musa leaves (250 mg/kg)	3.25	2.62	933.75± 10 76$^{@*}$	15.11±0.97*
Musa leaves (500 mg/kg)	2	1.37	788.57±8.85$^{@*\#}$	28.31±0.8$^{*\#}$
Musa pseudostem (250 mg/kg)	2	1.62	786.25±8.64$^{@*\#}$	28.52±0.78$^{*\#}$
Musa pseudostem (500 mg/kg)	1.87	1.25	656.25±9.89$^{@\#\$\&}$	40.34±0.89$^{\#\$\&}$
Musa fruits (250 mg/kg)	1.37	0.87	647.5±7.5$^{@\#\$\&}$	41.13±0.68$^{\#\$\&}$
Musa fruits (500 mg/kg)	0.77	0.55	513.33±5.59$^{@*\#\$\ !\ €}$	53.33±0.38$^{*\#\$\&!\ €}$

Results are expressed as means of ulcer number,severity,index and % of treatment protective effect± SE after fourteen days of Musa extract and prednisolone adminstration.followed by ulcer induction by single pr acetic acid infusion. n = 8; Data were analysed using one way analysis of variance (ANOVA) followed by Tukey Kramer's multiple comparison test; Significance was considered at $P \leq 0.05$

@Significantly different from positive control group, *Significantly different from prednisolone group,

Significantly different from Musa leaves 250 mg/kg group,$ Significantly different from Musa leaves 500 mg/kg group,& Significantly different from Musa pseudostem 250 mg/kg group,! Significantly different from Musa pseudostem 500 mg/kg group, € Significantly different from Musa fruits250 mg/kg group.

Table 8.Pathologic scoring of colon tissue damage assessed in the normal and treated groups.

Group	Histopathologic lesion scoring (Mean±SD)	Histopathologic lesion scoring (Mean±SE)
Normal	0.10±0.31	0.10±0.31
Positive Control (C+ve) Acetic acid 8%(2ml/rat)	5.00^{a}±0.00	5.00^{a}±0.00
Prednisolone	1.00^{d}±0.94	1.00^{d}±0.94
Musa leaves (250 mg/kg)	4.80^{a}±0.42	4.80^{a}±0.42
Musa leaves (500 mg/kg)	3.20^{b}±0.78	3.20^{b}±0.78
Musa pseudostem (250 mg/kg)	2.30^{c}±0.82	2.30^{c}±0.82
Musa pseudostem (500 mg/kg)	$1.90^{b,c}$±1.28	$1.90^{b,c}$±1.28
Musa fruits (250 mg/kg)	$1.50^{c,d}$±1.17	$1.50^{c,d}$±1.17
Musa fruits (500 mg/kg)	$0.80^{d,e}$±0.63	$0.80^{d,e}$±0.63

Table 9. MPO expression recorded in the colonic mucosa and submucosa of normal and treated groups.

Group	MPO expression in the colonic mucosa (% of positive cells/HPF) (Mean±SD)	MPO expression in the colonic mucosa (% of positive cells/HPF) (Mean±SE)	MPO expression in the colonic submucosa (% of positive cells/HPF) (Mean±SD)	MPO expression in the colonic submucosa (% of positive cells/HPF) (Mean±SE)
Negative control	0.20^{d}±0.42	0.20^{d}±0.13	0.10^{d}±0.31	0.10^{d}±0.10
C+ve Acetic acid 8% (2ml/rat)	3.30^{a}±0.48	3.30^{a}±0.15	3.90^{a}±0.31	3.90^{a}±0.10
Prednisolone	$0.80^{c,d}$±0.63	$0.80^{c,d}$±0.20	$0.80^{c,d}$±0.42	$0.80^{c,d}$±0.13
Musa leaves (250 mg/kg)	3.30^{a}±0.67	3.30^{a}±0.21	3.40^{b}±0.69	3.40^{b}±0.22
Musa leaves (500 mg/kg)	2.20^{b}±1.13	2.20^{b}±0.35	1.30^{c}±0.67	1.30^{c}±0.21
Musa pseudostem (250 mg/kg)	1.10^{c}±0.73	1.10^{c}±0.23	$1.10^{b,c}$±0.73	$1.10^{b,c}$±0.29
Musa pseudostem (500 mg/kg)	0.90^{c}±0.56	0.90^{c}±0.17	$0.70^{c,d}$±0.40	$0.70^{c,d}$±0.15
Musa fruits (250 mg/kg)	1.00^{c}±0.47	1.00^{c}±0.14	$1.00^{b,c}$±0.66	$1.00^{b,c}$±0.21
Musa fruits (500 mg/kg)	$0.50^{c,d}$±0.52	$0.50^{c,d}$±0.16	0.20^{d}±0.42	0.20^{d}±0.13

Table10.CRP and ILβ6 anti-inflammatory activity of Musa leaves, pseudo-stem and fruit peels.

Group (Parameter)	CRP (ng/ml)	ILβ6 (pg/ml)
Negative Control	1.36±0.08	43.83±1.86
Positive Control group Acetic acid 8% (2ml/rat)	$3.07\pm0.11^{@}$	$114.3\pm2.62^{@}$
Reference group Prednisolone (25 mg/kg)	$2.18\pm0.01^{@*}$	$54.5\pm0.51^{*}$
Musa leaves extract (250mg/kg)	$2.64\pm0.04^{@\$}$	$80.58\pm5.37^{@*\$}$
Musa leaves extract (500mg/kg)	$2.61\pm0.13^{@}$	$69.43\pm2.82^{@*}$
Musa pseudostem extract (250mg/kg)	$2.44\pm0.23^{@*}$	$70.3\pm3.58^{@*\$}$
Musa pseudostem extract (500mg/kg)	$2.15\pm0.17^{@*}$	$63.7\pm2.16^{@*}$
Musa fruits extract (250mg/kg)	$2.57\pm0.13^{@}$	$60.57\pm0.99^{@*}$
Musa fruits extract (500mg/kg)	$1.88\pm0.16^{*}$	$56.78\pm2.75^{*}$

Results are expressed as mean of levels of CRP and IL β6 ± S.E in serum of rats treated with Musa leaves ,pseudo-stem and fruits(250 and 500 mg/kg) and Prednisolone (2.8 µmol /kg) for two successive weeks followed by induction of colon lesions by using acetic acid 8%(2ml/rat); n = 8; Data were analysed using one way analysis of variance (ANOVA) followed by Tukey Kramer's multiple comparisons test; Significant at P ≤ 0.0001

@ Significant different from negative control;*Significant difference from positive control group; group;$ Significant difference from prednisolone group.

Figures

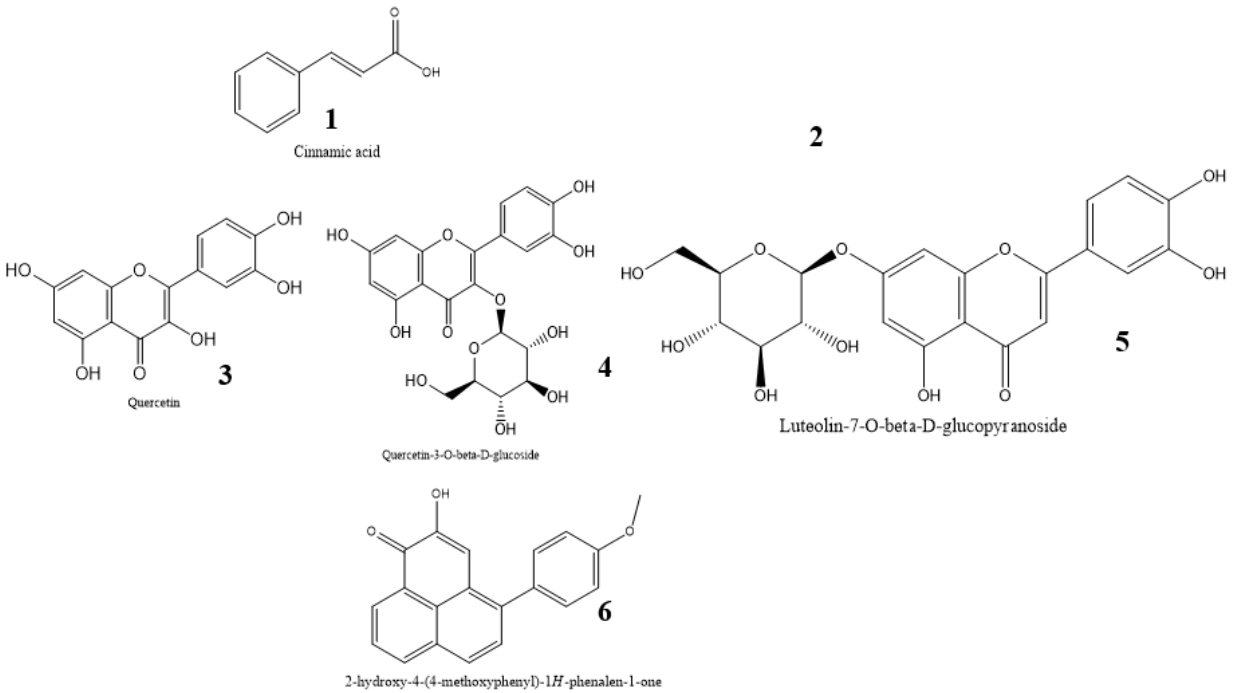

Figure 1

Six isolated compounds two phenolic acids, one phenylphenalenone and three flavonoids.

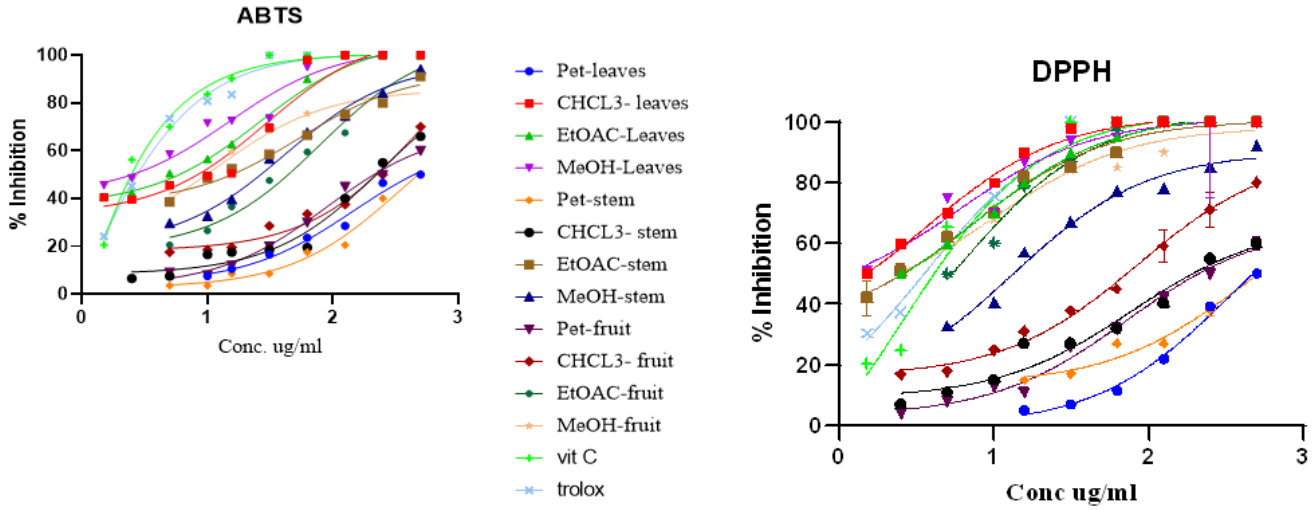

Figure 2

In vitro IC_{50} DPPH and ABTS antioxidant activity of different plant parts of *Musa acuminate* extracts.

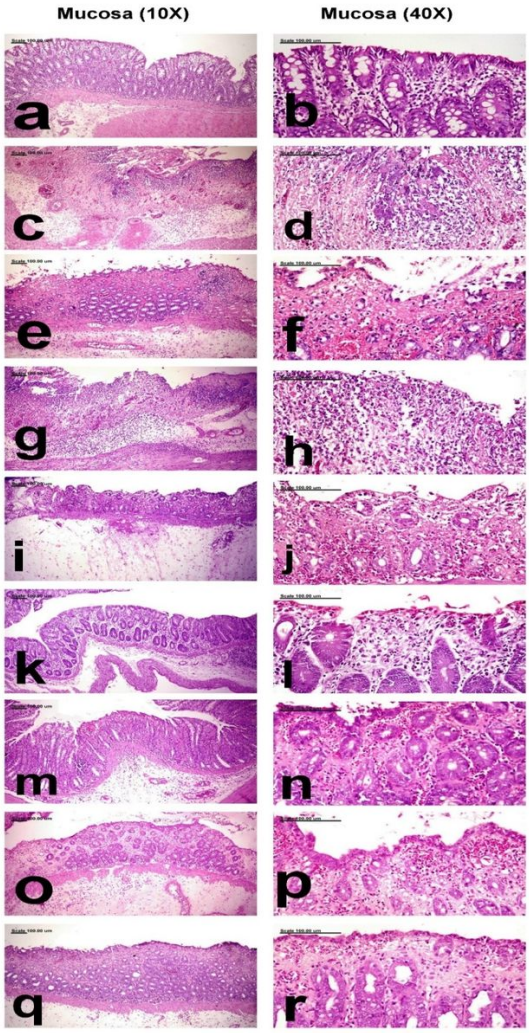

Figure 3

photomicrograph from the colon mucosa of, (a,b) negative control rats showing normal mucosa (a) and normal lamina propria containing few eosinophils as well as normal crypts lined by mucin-secreting cells (b), (c,d) C+ve showing diffuse ulcerative colitis with diffuse necrosis and desquamation of mucosal epithelium and crypts which are intensely infiltrated by neutrophils (c) in addition to severe congestion of mucosal blood vessels and aggregation of bacterial colonies (d), (e,f) Prednisolone treated group showing small focal ulcerative lesions with focal necrosis and desquamation of mucosal epithelium (e) and few proprial hemorrhage (f), (g,h) Leave (250 mg/kg)group showing diffuse necrosis of colonic mucosa associated with severe congestion of mucosal blood vessels (g) and massive neutrophilic cell infiltration(h) (I,j) the Leave (500mg/kg)group showing large focal erosive lesion (i) and few proprial hemorrhage (j), (k,l) Stem (250 mg/kg) group showing normal mucosal epithelium (k) and mild proprial edema as well as few leukocytic cell infiltration, (m,n) Stem (500 mg/kg) group showing regeneration of the mucosal epithelium (m) and minimal leukocytic cell infiltration as well as scant proprial hemorrhage (n), (o,p) Fruit (250 mg/kg) treated group showing regeneration of mucosal epithelium (o) and scant proprial hemorrhage (p), and (q,r) Fruit (500 mg/kg) treated groups showing normal colonic mucosa (q) and scant proprial hemorrhage (r). (Stain:H&E; Scale bar=100µm).

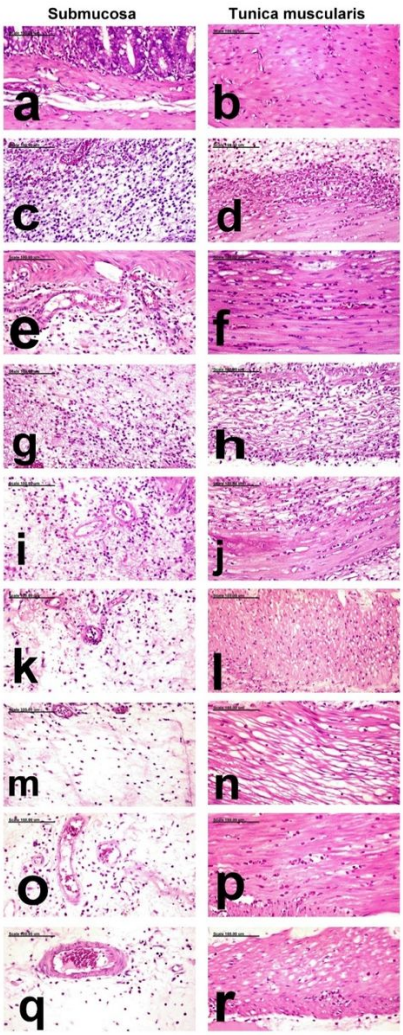

Figure 4

photomicrograph from the colon submucosa and T.muscularis of, (a,b) negative control rats showing normal submucosa (a) and T.muscularis (b), (c,d) C+ve showing expansion of the submucosa (c) and T.muscularis (d) by edematous fluids and intense neutrophilic cell infiltration, (e,f) Prednisolone treated group showing congestion of the submucosal blood vessels and infiltration of the submucosa (e) and T.muscularis (f) with few neutrophils, (g,h) Leave (250 mg/kg)group showing intense infiltration of the submucosa with neutrophils (g) and marked separation of muscle fibers of T.muscularis by edematous fluid and leukocytic cell infiltration (h), (I,j) the Leave (500mg/kg)group showing intense infiltration of the sub mucosa (i) and T.muscularis (j) with neutrophils, (k,l) Stem (250 mg/kg) group showing mild infiltration of submucosa (k) and T.muscularis (l) with neutrophils, (m,n) Stem (500 mg/kg) treated group showing few neutrophils infiltrating the submucosa (m) and T.muscuaris (n), (o,p) Fruit (250 mg/kg) treated group showing mild infiltration of the submucosa (o) and T.muscularis (p) with neutrophils, and (q,r) Fruit (500 mg/kg) treated group showing Sparse neutrophils in the submucosa (q) and T.muscularis (r). (Stain:H&E; Scale bar=100µm).

Figure 5

Photomicrograph from the liver of, (a) negative control rats showing normal hepatic parenchyma with normal hepatocytes, (b) C+ve showing mild granular degeneration of hepatocytes, (c) Prednisolone treated group showing mild focal vacuolar degeneration of hepatocytes, (d) the low dose leave group showing swelling and vacuolation of hepatocellular cytoplasm, (e) the high dose leave group showing normal hepatocytes, (f) low stem-treated groups showing normal histological structures, (g) high dose stem-treated groups showing normal hepatocytes, (h) low dose fruit treated group showing mild focal congestion of some hepatic sinusoids, and (i) high dose fruit treated groups showing normal hepatic parenchyma. (Stain:H&E; Scale bar=100μm).

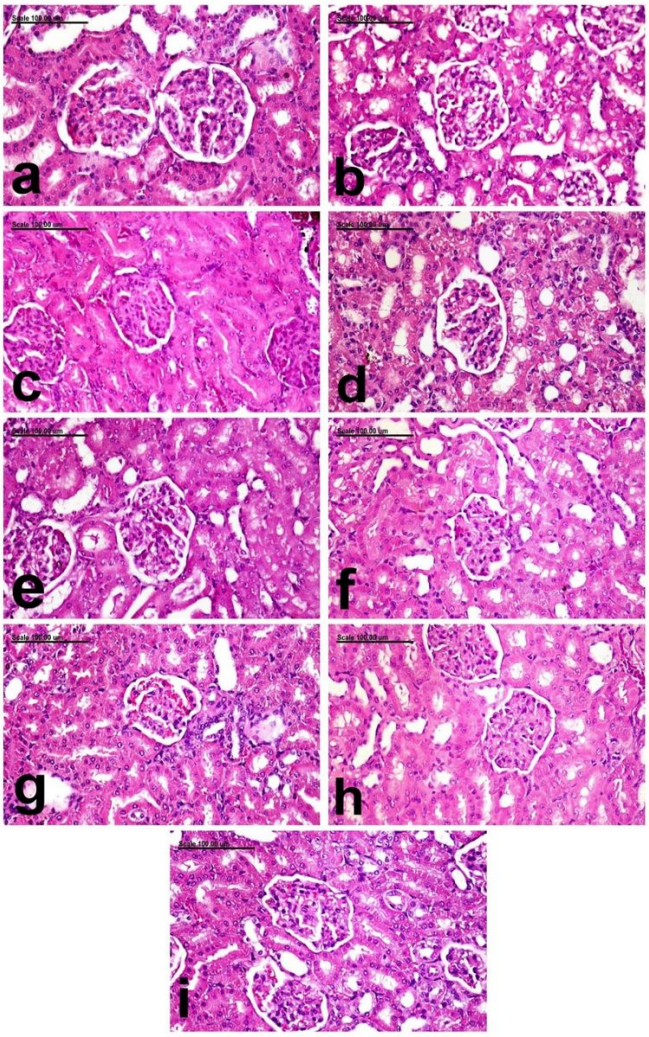

Figure 6

photomicrograph from the kidneys of, (a) negative control rats showing normal renal tubules and glomeruli, (b) C+ve showing vacuolation of individual cells lining the renal tubules, (c) Prednisolone treated group showing normal renal tubules, (d) the low dose leave group showing vacuolization of some renal tubular epithelial cells, (e) the high dose leave group showing normal renal tubules, (f) low stem-treated groups showing normal histological structures, (g) high dose stem-treated groups showing normal renal parenchyma, (h) low dose fruit treated group showing normal histological structures, and (i) high dose fruit treated groups showing normal renal parenchyma. (Stain:H&E; Scale bar=100μm).

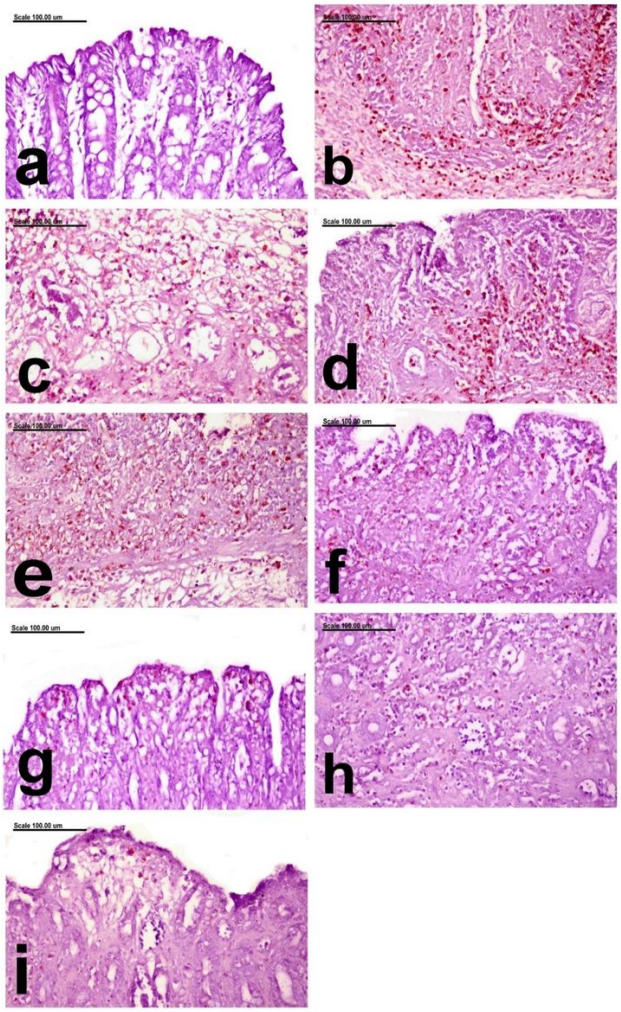

Figure 7

photomicrograph from the MPO immunohistochemically-colon mucosa of, (a) negative control rats showing Individual MPO+ cells in the mucosa, (b) C+ve group showing increase of % of MPO+ cells in the colonic mucosa, (c) Prednisolone treated group showing significant decrease of % of MPO+ cells, (d) the low dose leave group showing increased % of MPO+ cells, (e) the high dose leave group showing decteased % of MPO+ cells, (f) low stem-treated groups showing marked decrease of % of MPO+ cells, (g) high dose stem-treated groups showing significant decrease of % of MPO+ cells, (h) low dose fruit treated group showing remarkable decrease of MPO+ cells, and (i) high dose fruit treated groups showing few scattered MPO+ cells.(MPO immunohistochemical staining \; Scale bar=100μm).

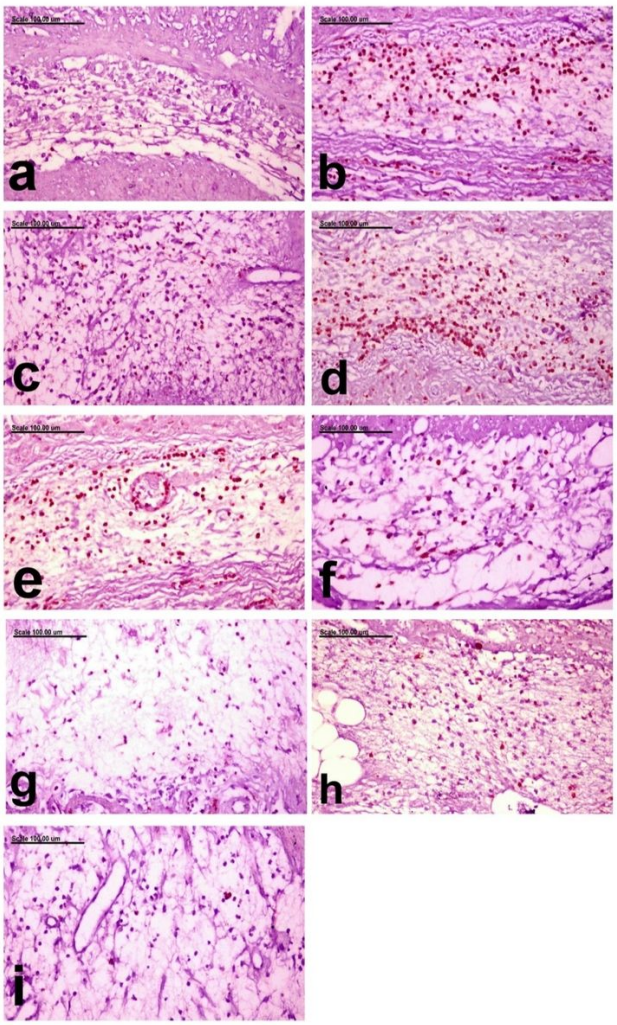

Figure 8

photomicrograph from the MPO immunohistochemically-colon submucosa of, (a) negative control rats showing Individual brown stained MPO+ cells in the mucosa, (b) C+ve rats showing numerous intensely brown stained MPO+ cells in the colonic submucosa and T.muscularis, (c) Prednisolone treated group showing significant decrease of brown stained MPO+ cells, (d) the low dose leave group showing abundant brown stained MPO+ cells, (e) the high dose leave group showing brown stained MPO+ cells in the wall of the submucosal blood vessel and the submucosa, (f) low stem-treated groups showing marked decrease of % of MPO+ cells, (g) high dose stem-treated groups showing remarkable decrease of % of MPO+ cells, (h) low dose fruit treated group showing significant decrease of MPO+ cells with brown staining, and (i) high dose fruit treated groups showing few scattered MPO+ cells. (MPO immunohistochemical staining \; Scale bar=100μm).

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