Advances in Traditional Medicine (2024) 24:459–474
https://doi.org/10.1007/s13596-023-00708-z

RESEARCH ARTICLE

Argania spinosa essential oil ameliorates colonic damage and extraintestinal alterations in a rat model of acetic acid-induced colitis by suppressing oxidative stress and inflammation

Folake Olayinka Olojo[1] · Akinleye Stephen Akinrinde[2] · Stella Ajedawun Ogundairo[1] · Vincetia Chinwendu Ubochi[1]

Received: 13 April 2023 / Accepted: 3 September 2023 / Published online: 27 September 2023

Abstract

The present study was designed to elucidate the prophylactic and therapeutic potential of argan oil (AO) (from the kernels of the argan tree, *Argania spinosa*) against acetic acid (AA)-induced colitis and associated alterations in the liver and kidneys of rats. Colitis was induced by intra-rectal administration of 4% AA solution for 3 consecutive days. Some groups of rats were treated orally with AO (5 mL/kg) for 5 consecutive days before and after AA administration, while other groups were treated with either the vehicle or AO alone. Macroscopic and microscopic lesions in the tissues were assessed, while oxidative stress, antioxidant parameters and myeloperoxidase (MPO) activity were determined by biochemical methods. Haematological and serum chemistry parameters were also evaluated. Administration of AO before or after AA induction produced improvements in body weight gain, faecal consistency, macroscopic and histologic scores of the colonic mucosa compared to rats treated with AA alone. Furthermore, AO treatment caused significant reduction in colonic levels of hydrogen peroxide (H_2O_2), malondialdehyde (MDA), advanced oxidation protein products (AOPP) and serum MPO activity, while glutathione S-transferase (GST) and superoxide dismutase (SOD) activities were increased in the colon and kidneys, compared to the colitis control. Acetic acid treatment resulted in significant reduction in erythrocyte and leucocyte indices in relation to healthy controls. Taken together, treatment of rats with AO protected colonic tissues from acetic acid-induced inflammation and suggests that the oil may be considered for preventive and therapeutic purposes against inflammatory bowel diseases.

Keywords *Argania spinosa* · Acetic acid · Inflammation · Oxidative stress · Colitis

Introduction

Ulcerative colitis (UC), a subtype of the wider class of inflammatory bowel diseases (IBD), is a chronic, often refractory inflammation of the mucosa of the large intestine (colon and rectum). The etiology of UC is not completely clear but is thought to be related to abnormalities in the regulation of mucosal immunity against resident potentially pathogenic bacteria in the gut leading to persistent cytokine production along with contributory pathogenetic roles of genetic susceptibility and environmental factors (Podolsky 2002; Rezayat et al. 2018). In addition, reactive oxygen species are believed to play a significant role in the pathogenesis of UC (Cagin et al. 2016). Experimentally, induction of UC via intra-rectal administration of acetic acid (AA) has been severally employed as a suitable reproducible animal model which presents characteristic features of IBD such as colonic inflammation, intestinal scarring and fibrosis, diarrhea, lipid

Folake Olayinka Olojo and Akinleye Stephen Akinrinde have equally contributed to this work.

✉ Folake Olayinka Olojo
flackey2008@gmail.com; folakemiyinka@yahoo.com

✉ Akinleye Stephen Akinrinde
as.akinrinde@ui.edu.ng; as.akinrinde@gmail.com

[1] Department of Chemistry, Faculty of Science, The Polytechnic, Ibadan, Oyo State, Nigeria

[2] Gastrointestinal and Environmental Toxicology Laboratory, Department of Veterinary Physiology and Biochemistry, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Oyo State, Nigeria

peroxidation and oxidative stress (Qin et al. 2011, Kumar et al., 2013).

More recently, greater attention has also been focused on extra-intestinal manifestations of inflammatory bowel diseases which may involve several other organ systems and thus worsen the disease burden on the patient, while also creating a huge challenge to the physician (Levine and Burakoff 2011). Such extra-colonic lesions have been observed in organs such as the liver including primary sclerosing cholangitis, portal vein thrombosis and drug-induced hepatotoxicity (Lee and Kaplan 1995; Lapidus et al. 1999; Mohamed et al. 2021). Renal effects such as proteinuria, renal failure, and uremia have also been found as complications in patients with IBD (Wester et al. 2001). Furthermore, blood related-diseases such as iron deficiency anaemia, caused by apparent or occult gastrointestinal bleeding and autoimmune haemolytic anaemia are among extra-intestinal complications of UC (Imagawa 1999; Gasche et al. 2004).

Complete remission of UC is very difficult to achieve and as a result, currently available therapeutic strategies including corticosteroids and 5-aminosalicylate are mainly targeted at improving the quality of life of the patient by promoting healing of the colonic mucosa and achieving clinical remission in order to eliminate the need for surgical management (Ke et al. 2012). The use of herbal remedies in the management of UC has thus intensified in recent years due to their perceived potentially greater efficacy and safety over commonly used synthetic drugs such as azathioprine, cyclosporine, 6-mecaptopurine and 5-aminosalicylate (Yadav and Liu 2009). Essential oils have attracted significant attention in recent years because of their application as pharmaceutical agents in the treatment of several diseases (Shaaban et al. 2012; Rezayat et al. 2018). The potential therapeutic efficacy of essential oils against UC could derive from a blend of several biologically active constituents with reported anti-inflammatory properties such as the down-regulation of pro-inflammatory cytokine gene expression and reduction in the activities of pro-inflammatory enzymes such as myeloproxidase (MPO), cyclooxygenases (COX) and nitric oxide synthase (iNOS) (Basholli-Salihu et al. 2017).

Argan oil (AO) is extracted from portions of the tree *Argania spinosa*, which is native to parts of northern Africa including Morocco, Tunisia and Algeria. The oil is consumed largely for its cardioprotective properties and benefits in treatment of skin infections, although several other potential health benefits are yet to be researched (Hanana et al. 2018; Menni et al., 2019). Its associated biological and nutraceutical effects include anti-inflammatory, antioxidative and antiproliferative activities which are thought to be mediated by the actions of major constituents such as mono- and poly-unsaturated fatty acids, as well as minor components including polyphenols, tocopherols and sterols (Monfalouti et al. 2010). Studies on healing of colonic mucosa following experimental colorectal anastomosis in rats revealed that intra-rectal application of argan oil caused improvement in colonic wound healing parameters in the colon due to its antioxidant and anti-inflammatory effects (Barlas et al. 2018). Furthermore, the anti-inflammatory potential of argan oil has recently been reported in a carrageenan-induced rat model of inflammation (Menni et al. 2020).

In view of its reported health benefits, the present study was aimed to evaluate the possible therapeutic and/or prophylactic effects of argan oil on oxidative tissue damage and colonic inflammation in a rat model of experimental colitis induced by acetic acid.

Materials and methods

Animals

This study was conducted using male Wistar rats, about 8 weeks of age and weighing between 160 and 180 g. They were obtained from the Experimental Animal Unit, Faculty of Veterinary Medicine, University of Ibadan, Nigeria. The experiment was conducted in an animal house facility where rats were housed in plastic cages and were initially acclimatized for one week. The rats were maintained under controlled environmental conditions (22 ± 2 °C and a 12 h dark/12-h light photoperiod cycle) and were allowed free access to feed and clean tap water throughout the experimental period. This study was conducted according to guidelines outlined in the National Institute of Health publication, "Guide for the Care and Use of Laboratory Animals" (PHS, 1996) and also followed protocols approved by the Animal Care and Use Research Ethics Committee (*UI-ACUREC/411120/13*) of the University of Ibadan.

Experimental design and induction of colitis

Thirty male Wistar rats were randomly assigned to five groups of six rats each and were treated as follows (Table 1):

Group A Control; 1 mL normal saline (vehicle) administered intra-rectally for 3 days
Group B Acetic acid (1 mL of 4% acetic acid solution administered via a 6G catheter inserted 6 cm into the anus, after which rats were maintained in the Trendelenburg position for 15 min. Administration was done at 24 h interval for 3 days. Colitis induction was guided by protocols described in previous studies (Cetinkaya et al. 2006; Kumar et al. 2013; Cagin et al. 2016)
Group C 4% Acetic acid (1 mL per day intra-rectally) for 3 days + post-treatment with Argan oil (5 mL/kg) administered by oral gavage from day four, for 5 days.

Table 1 Experimental design

Groups	Days								
	1	2	3	4	5	6	7	8	9
A						NS	NS	NS	Sacrifice
B						AA	AA	AA	
C	AA	AA	AA	AO	AO	AO	AO	AO	
D	AO	AO	AO	AO	AO	AA	AA	AA	
E				AO	AO	AO	AO	AO	

NS normal saline, *AA* acetic acid, *AO* Argan oil

Group D Rats pre-treated with Argan oil (5 mL/kg) for 5 days followed by 4% Acetic acid (1 mL per day intrarectally) for 3 days.
Group E Rats given Argan oil alone (5 mL/kg) for 5 days.

Body weight was recorded for each animal daily up till the termination of the experiment. On the 9th day, stool consistency was graded as 1: watery; 2: semi-solid/pasty and 3: solid/normal. Thereafter, blood was withdrawn from the retro-orbital plexus for haematology and serum chemistry analyses and the rats were then sacrificed by cervical dislocation. The colon, liver and kidneys from each animal were dissected and weighed immediately. The length of the colon from each rat was also measured with the aid of a graduated ruler.

Macroscopic and histopathological assessment of tissue damage

Freshly dissected colon from rats were opened along their entire length and examined macroscopically for features of colitis induction. Thereafter, small pieces of the colon, liver and kidneys were immediately transferred into 10% phosphate buffered saline for histopathological studies. Briefly, the tissues were embedded in paraffin followed by sectioning into tissue slices of about 4–5 µm. The sections were later mounted on plain glass slides, deparrafinized and stained with haematoxylin and eosin (H&E). The morphology of the tissues was examined and photomicrographs were captured at magnifications of 100× and 400× with aid of a light microscope and digital camera (Drury et al. 1976).

Biochemical analyses

Colon, liver and kidney tissues were homogenized in six parts of homogenizing buffer (potassium phosphate buffer containing 1.15% KCl (pH 7.4). The homogenates were centrifuged at 10,000 g for 10 min. at 4 °C using a refrigerated centrifuge and the supernatant was extracted as post-mitochondrial fraction for the analyses of biochemical parameters of oxidative stress and antioxidant status.

Assessment of tissue oxidative and inflammatory status

Hydrogen peroxide (H_2O_2) concentration in the homogenates was determined spectrophotometrically according to the method of Wolff (1994). The reaction mixture was prepared in microtitre plates and consisted of 100 µL phosphate buffer (0.1 M; pH 7.4), 50 µL ammonium ferrous sulphate, 20 µL sorbitol, 20 µL xylenol orange and 10 µL sulphuric acid added to 10 µL of the homogenate. The mixture was thoroughly vortexed and incubated at room temperature for 30 min. and the absorbance was read at 560 nm using a Microplate Reader.

Lipid peroxidation was quantified spectrophotometrically as the concentration of malondialdehyde (MDA) in the tissue homogenates (Varshney and Kale 1990). Briefly, 0.8 mL of Tris-Kcl buffer (0.15 M; pH 7.4) was added to 0.2 mL of the homogenate in a test tube followed by 0.25 mL of 30% tricholoroacetic acid and 0.25 mL of 0.75% thiobarbituric acid. The mixture was heated in a water bath set at 80 °C for 45 min. Thereafter, the reaction mixture was centrifuged and 200 µL of the supernatant was placed in microtitre plates from where the absorbance was read at 532 nm using a Microplate Reader. The value of MDA was calculated using a molar extinction coefficient of 1.56×10^5 m^{-1} cm^3.

Protein oxidation was assessed by measuring the level of advanced oxidation protein products (AOPP) in the tissues according to the methods described by Kayali et al (2006). The activity of myeloperoxidase (MPO) in the colon was used as an index of tissue inflammation and was estimated according to the method of Xia and Zweier (1997).

Assessment of tissue antioxidant status

The content of reduced glutathione in the homogenates was measured according to the method of Beutler et al. (1963). Briefly, 100 µL of the homogenate was deproteinized in equal volume of a precipitating solution (4% sulfosalicylic acid). The mixture was centrifuged at 4000 rpm for 5 min after which 20 µL of the supernatant was added to 180 µL of Ellman's reagent (5,5′ Dithio-bis-(2-nitrobenzoic acid prepared in 0.1 M phosphate buffer) in microtitre plates with

the test performed in duplicates for each sample. The absorbance was read at 412 nm using a Microplate reader.

The activity of Glutathione S-transferase was determined by methods described by Habig et al. (1974). The assay involved a kinetic measurement of the degree of conjugation of GSH with 1-choloro-2,4-dibenbenzene (CDNB) as second substrate. The homogenates were assayed in a medium containing 140 µL of phosphate buffer (0.1 M; pH 6.5), 10 µL of sample, reduced glutathione (10 µL) and CDNB (50 µL) in well of a microtitre plate. The absorbance of the mixture was monitored over 3 min in 30 s-intervals at a wavelength of 340 nm.

Superoxide dismutase (SOD) activity was measured from its ability to inhibit the auto-oxidation of adrenaline according to the method of Misra and Fridovich (1972). The assay medium consisted of sample (30 µL) added to sodium carbonate buffer (2.5 mL; pH 10.2) and adrenaline (300 µL) in a test tube. The mixture was immediately transferred to a cuvette in a UV–Vis spectrophotometer and the increase in absorbance at 340 nm was monitored every 30 s for 150 s.

Haematology and serum chemistry evaluation

Whole blood samples were employed for the evaluation of haematological indices including packed cell volume (PCV), haemoglobin concentration (Hb), red blood cell count (RBC), white blood cell count (WBC) and platelet count using an auto haematology analyzer (SYSMEX Automated Haematology Analyser, United Kingdom). Other blood samples were centrifuged at 3000 rpm for 10 min to obtain the serum as supernatant. The serum was used for the assessment of biochemical parameters including total protein, albumin, globulin, electrolytes (Na^+, K^+, Cl^- and HCO_3^-), urea creatinine, alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT) and bilirubin (total and conjugated).

Gas chromatography-mass spectrometry (GC–MS) analysis of Argan oil

GC–MS analysis of the oil was performed using an Agilent 5977B GC/MSD system coupled with Agilent 8860 auto-sampler, a Gas Chromatograph interfaced to a Mass Spectrometer (GC–MS) equipped with an Elite-5MS (5% diphenyl/95% dimethyl polysiloxane) fused to a capillary column (30 × 0.25 µm ID × 0.25 µm df). For GC–MS detection, an electron ionization system was operated in electron impact mode with ionization energy of 70 eV. Helium gas (99.999%) was used as a carrier gas at a constant flow rate of 1 mL/min, and an injection volume of 1 µL was employed (a split ratio of 10:1).

The injector temperature was maintained at 300 °C, and the ion-source temperature was 250 °C, and the oven temperature was programmed from 100 °C (isothermal for 0.5 min), with an increase of 20 °C/min to 280 °C (2.5 min), Mass spectra were taken at 70 eV; a scanning interval of 0.5 s and fragments from 45 to 450 Da. The solvent delay was 0–3 min, and the total GC/MS running time was 21.33 min. Interpretation of mass spectrum GC–MS was conducted using the database of the National Institute Standard and Technology (NIST) having more than 62,000 patterns and the National Center for Biotechnology Information. The spectrum of the unknown components was compared with the spectrum of known components stored in the NIST library.

Statistical analysis

The data were expressed as mean ± standard deviation. The differences in group means were statistically compared using One-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons. *P* values less than 0.05 were recorded as statistically significant. Statistical analysis was performed using GraphPad Prism software (version 7.00).

Results

Effect of argan oil on body weights, organosomatic indices and stool consistency in acetic acid-induced colitis

Intra-rectal administration of acetic acid resulted in progressive decline in body weight from the day following the first administration until the last day (Fig. 1). Overall, rats treated with acetic acid alone experienced loss of weight

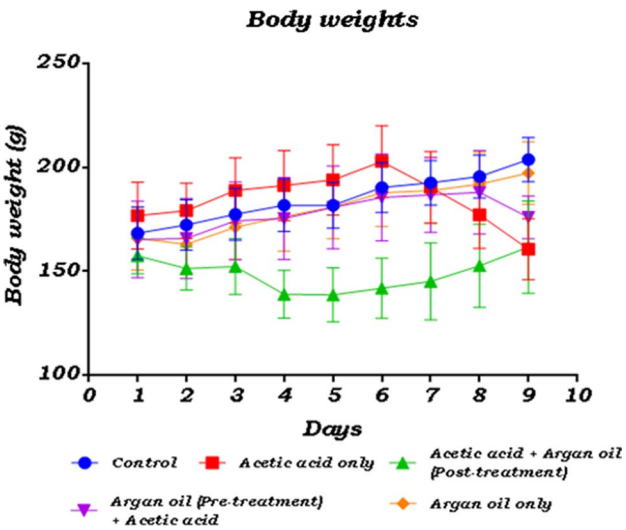

Fig. 1 Daily body weight changes during the experimental period

(− 10.1%) over the course of the experiment (Table 2). In contrast, the average weights of control rats increased progressively throughout the duration of the experiment, with an average weight gain of 17.42% (Table 2). Similar progressive increase in body weight (15.96%) was observed in rats treated with Argan oil alone. Rats undergoing post-treatment with Argan oil after initial acetic acid treatment recorded a corresponding initial decrease in body weight which subsequently improved upon Argan oil treatment. This group of rats had a marginal increase in percentage body weight gain of 2.58% over the course of the experiment (Table 2). Rats pre-treated with Argan oil showed an initial increase in body weights, followed by a sharp decline corresponding to the commencement of acetic acid administration. This latter group of rats showed a moderate increase (6.1%) in weight gain at the end of the experiment.

Compared to the control, rats given intra-rectal acetic acid alone showed significant ($P < 0.05$) increase in wet weight and relative weight of the colon, while pre- or post-treatment with Argan oil resulted in amelioration of the acetic acid-induced increase in colon weight (Table 2). Treatment of rats with Argan oil alone also caused significant reduction in the wet weights of the colon compared to the acetic acid-treated group. There was significant ($P < 0.05$) reduction in colon lengths in the groups treated with acetic acid compared to the control rats. Consequently, the colon weight to length ratio was significantly increased in these groups compared to either the control or rats treated with Argan oil alone. There were no changes in both the absolute or relative weights of the liver and kidneys in all the experimental groups, indicating that the acetic acid insult did not impact significantly on organosomatic indices in these organs.

Rats treated with acetic acid alone generally passed watery faeces and thus recorded significantly ($P < 0.05$) lower stool consistency scores compared to the control rats and those treated with Argan oil which showed solid and semi-solid faeces, respectively (Table 2).

Effect of argan oil on colon macroscopic appearance

Macroscopically, the colonic mucosa of rats treated intrarectally with 4% acetic acid alone was characterized by key features of ulcerative colitis, including bleeding, necrosis, erosions, inflammation and ulceration (Fig. 2), all of which were absent in control rats and those treated with Argan oil alone. Similarly, argan oil treatment before or after induction of colitis suppressed the gross lesions induced by acetic acid.

Effect of argan oil on histologic alterations induced by acetic acid

The colonic mucosa of normal untreated rats had well preserved morphology with intact epithelial layer, crypts and glands and no inflammatory cell infiltration (Fig. 3A). However, in acetic acid-treated rats, the colonic mucosa presented histopathological features of ulcerative colitis including generalized necrosis of the mucosa, loss of the epithelial

Table 2 Effect of Argan oil on body weight change, organosomatic indices and stool consistency in acetic acid-induced colitis

	A	B	C	D	E
Initial body weight (g)	168.33 ± 12.66	176.83 ± 16.04	157.50 ± 8.71	165.33 ± 18.43	165.83 ± 15.22
Final body weight (g)	203.83 ± 10.69	160.67 ± 14.68	161.67 ± 22.21	176.00 ± 10.37	197.33 ± 15.10
% weight change	17.42	− 10.06	2.58	6.06	15.96
Colon weight (Abs) (g)	1.62 ± 0.50	1.86 ± 0.19[a]	1.47 ± 0.37	1.52 ± 0.23	1.26 ± 0.17
Colon weight (Rel) (g)	0.81 ± 0.24	1.15 ± 0.13[a]	0.89 ± 0.21	0.86 ± 0.14	0.63 ± 0.06[b]
Colon length (cm)	17.70 ± 2.10	11.88 ± 2.06[a]	12.00 ± 3.54[a]	12.10 ± 1.85[a]	14.70 ± 1.68
Colon weight to length ratio	0.09 ± 0.03	0.16 ± 0.05[a]	0.13 ± 0.04	0.13 ± 0.03	0.09 ± 0.01[b]
Liver weight (Abs) (g)	7.16 ± 0.42	6.38 ± 0.63	6.52 ± 0.71	6.99 ± 0.80	7.42 ± 1.49
Liver weight (Rel) (g)	3.81 ± 0.51	3.91 ± 0.25	3.998 ± 0.58	3.97 ± 0.63	3.71 ± 0.55
Kidney weight (Abs) (g)	1.57 ± 0.37	1.36 ± 0.07	1.23 ± 0.12	1.25 ± 0.11	1.48 ± 0.19
Kidney weight (Rel) (g)	0.77 ± 0.13	0.84 ± 0.10	0.75 ± 0.09	0.71 ± 0.10	0.74 ± 0.06
Stool consistency	3.00 ± 0.0	1.00 ± 0.0[a]	2.60 ± 0.55	2.00 ± 0.00	3.00 ± 0.0

Data are presented as mean ± standard deviation and means were compared by one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test

[a]Significant ($p < 0.05$) as compared to Group A

[b]Significant ($p < 0.05$) as compared to group B

[c]Significant ($p < 0.05$) as compared to group C

[d]Significant ($p < 0.05$) as compared to group D

Abs, absolute; Rel, relative

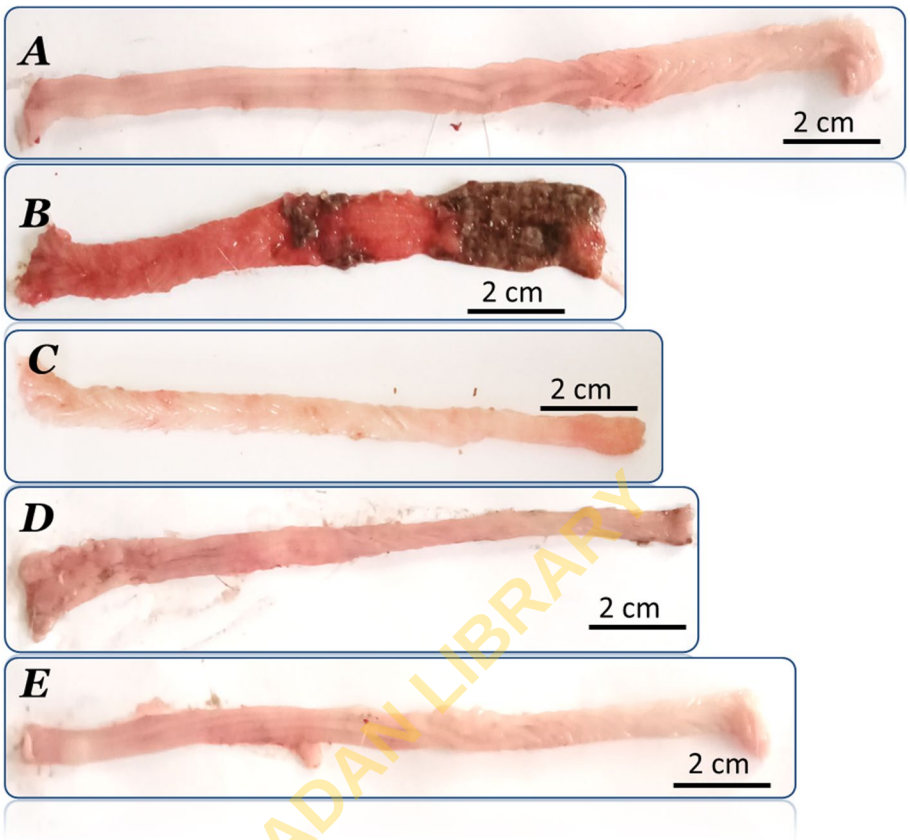

Fig. 2 Macroscopic appearance of colons. **A** Control, (**B**) acetic acid only, **C** Acetic acid + argan oil (post-treatment), **D** Argan oil (pre-treatment) + acetic acid, **E** Argan oil only

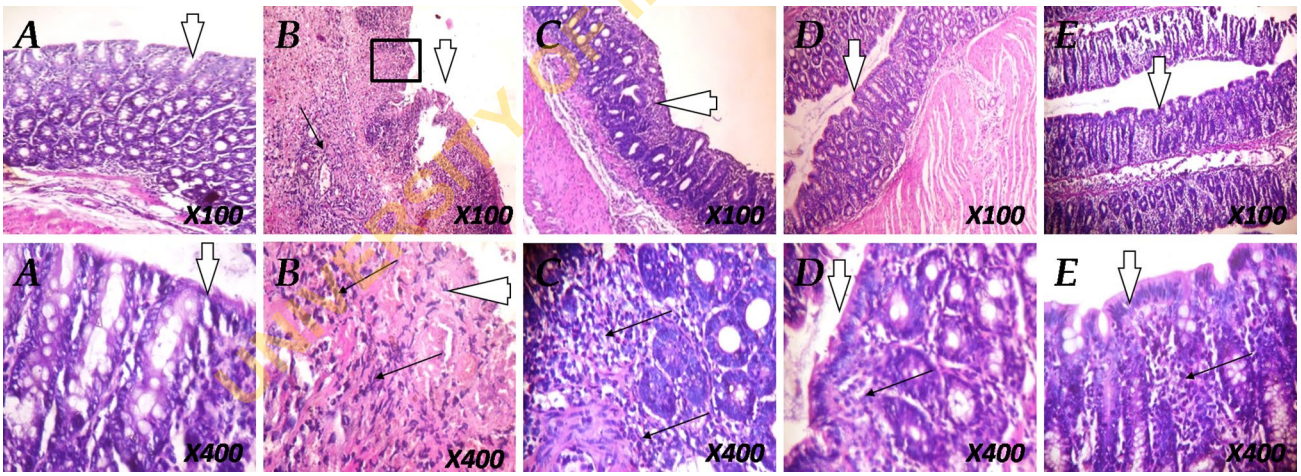

Fig. 3 Photomicrographs of rat colonic sections stained with H&E (Upper plates Mag. × 100; Lower plates Mag. × 400). **A** Control, **B** acetic acid only, **C** Acetic acid + argan oil (post-treatment), **D** Argan oil (pre-treatment) + acetic acid, **E** Argan oil only. The epithelium is indicated as white arrows while inflammatory cells are depicted as black arrows

layer and glands with inflammatory cell infiltration consisting of lymphocytes, polymorphs and plasma cells into the mucosa and submucosa. Furthermore, the circular muscles were no longer distinguishable due to necrosis (Fig. 3B). However, pre- or post-treatment of rats with Argan oil produced significant attenuation of the severity of histologic features of acetic acid colitis, with well-preserved colonic mucosa and only mild to moderate inflammatory cell infiltration (Fig. 3C, D). Rats treated with Argan oil alone also showed well preserved colonic morphology (Fig. 3E).

Liver tissues from acetic acid-treated rats showed moderate to severe congestion of the central venules, although

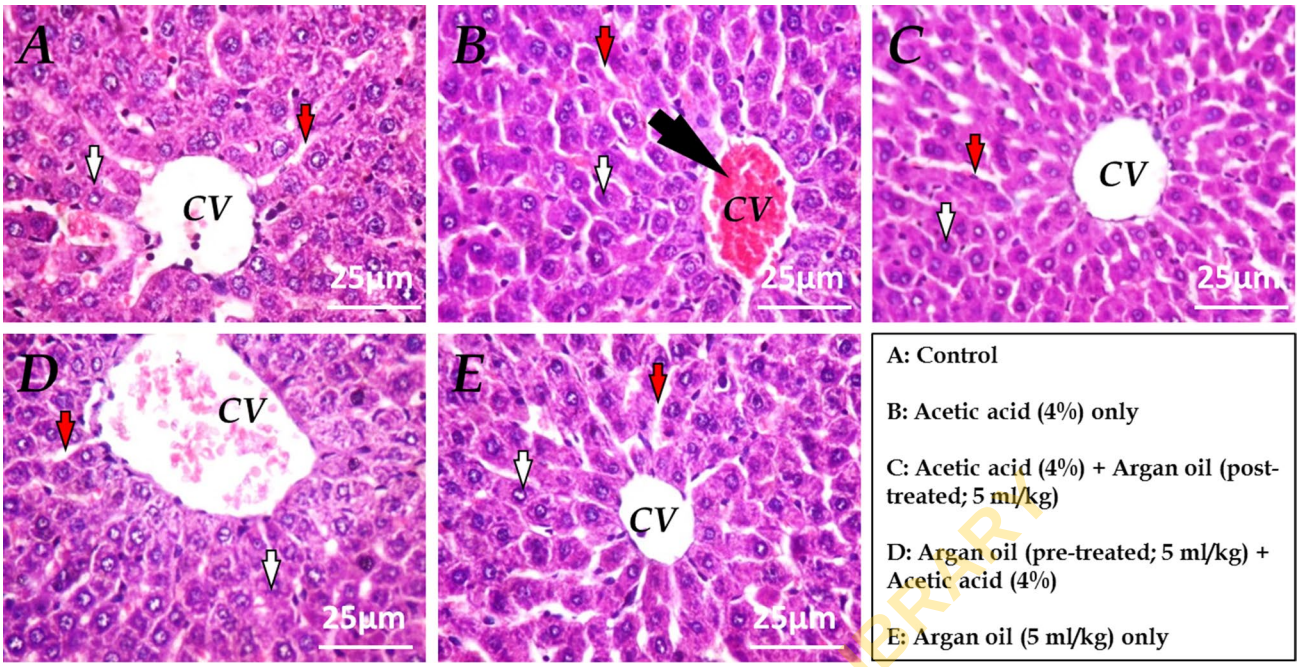

Fig. 4 Photomicrographs of rat liver sections stained with H&E (Mag. × 400). **A** Control, **B** acetic acid only, **C** Acetic acid + argan oil (post-treatment), **D** Argan oil (pre-treatment) + acetic acid, **E** Argan oil only. CV, central venules. Black arrow indicates congestion of the central venule; White arrows indicate hepatocytes; Red arrows show sinusoids

the morphology of the hepatocytes and sinusoids appeared normal (Fig. 4B). The morphology of the liver tissues from all other groups showed no significant lesions and absence of inflammatory cell infiltration (Fig. 4A, C–E).

Kidney sections from untreated controls showed normal architecture, with normal glomeruli, normal messangial cells, capsular spaces and renal tubules (Fig. 5A). However, kidney sections from acetic acid-treated rats presented poor architecture, including mild necrosis and sclerosis of glomeruli and congestion of renal tubules. The interstitial spaces also showed mild infiltration of inflammatory cells, while the messangial cells and capsular spaces still appeared normal (Fig. 5B). These lesions were ameliorated in rats treated with argan oil, although rats given Argan oil pre-treatment appeared to show a greater degree of improvement in tissue morphology compared to those that underwent post-treatment with Argan oil (Fig. 5C, D). Rats treated with argan oil alone had renal morphology that was comparable to those of the normal untreated rats (Fig. 5E).

Effect of argan oil on tissue hydrogen peroxide generation, lipid peroxidation and protein oxidation in acetic acid-induced colitis

Hydrogen peroxide (H_2O_2) concentration was significantly ($p < 0.05$) increased in colonic and hepatic tissues after intra-rectal acetic acid administration compared to normal rats. However, Argan oil pre-treatment or post-treatment resulted in significant ($p < 0.05$) attenuation of H_2O_2 generation in the colon, compared to rats that underwent treatment with acetic acid alone (Fig. 6). Rats treated with Argan oil alone also showed significantly ($p < 0.05$) lower H_2O_2 levels in the colon and kidneys compared to the acetic acid-treated rats.

Lipid peroxidation, indicated by tissue malondialdehyde (MDA) levels was significantly ($p < 0.05$) increased in the colon and liver following intra-rectal acetic acid treatment compared to the normal untreated rats (Fig. 6). Argan oil treatment either pre- or post-acetic acid treatment significantly ($p < 0.05$) inhibited the acetic acid induced increase in colonic MDA as compared to the rats treated with acetic acid alone. Similarly, rats treated with Argan oil alone exhibited significantly ($p < 0.05$) lower MDA values compared with the acetic acid-treated rats. Levels of MDA in the kidneys were, however, not significantly altered in all the groups of rats.

Protein oxidation, measured in this study as the concentration of advanced oxidation protein products (AOPP), was significantly ($p < 0.05$) increased in the colon and kidneys tissues of acetic acid-treated rats, compared to the untreated controls (Fig. 6). Treatment of rats with Argan oil before or after acetic acid induction led to significant ($p < 0.05$) reduction in AOPP levels in the colon. Similarly, treatment with Argan oil alone significantly ($p < 0.05$) inhibited protein oxidation in the colon and kidneys

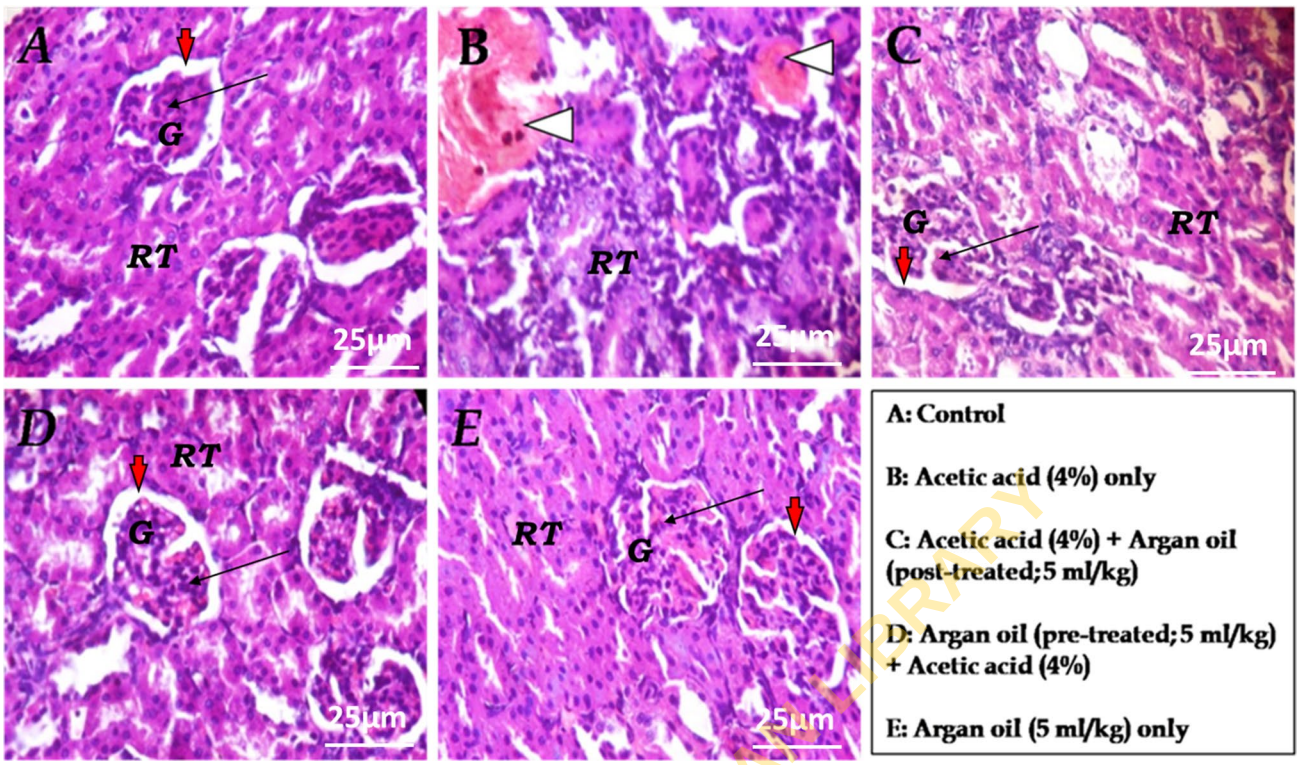

Fig. 5 Photomicrographs of rat kidney sections stained with H&E (Mag. × 400). **A** Control, **B** acetic acid only, **C** Acetic acid + argan oil (post-treatment), **D** Argan oil (pre-treatment) + acetic acid, **E** Argan oil only. White arrows indicate congested renal tubular spaces. *G*, glomerulus; *RT*, renal tubules. Red arrows show capsular spaces; black slender arrows indicate messangial cells

compared to the acetic acid control. Hepatic AOPP levels, however, remained unaltered across the different groups.

Effect of argan oil on colonic myeloperoxidase activity in acetic acid-induced colitis

Colonic myeloperoxidase ($p < 0.05$) activity was significantly ($p < 0.05$) elevated in rats undergoing acetic-acid colitis, as compared to the normal untreated rats (Fig. 7). In contrast, treatment of rats with Argan oil before or after acetic acid induction resulted in significant ($p < 0.05$) reduction in MPO activity compared to rats induced with acetic acid alone.

Effect of Argan oil on tissue antioxidant levels in acetic acid-induced colitis

Compared with the untreated controls, intra-rectal administration of acetic acid with or without argan oil treatment produced significant ($p < 0.05$) reduction in the levels of reduced glutathione (GSH) in the colon. Interestingly, rats treated with argan oil alone also showed reduced levels of colonic GSH. Nevertheless, GSH levels were unaltered in the liver and kidneys regardless of the treatment administered (Table 3).

Glutathione S-transferase (GST) activity was significantly ($p < 0.05$) inhibited in the colon, liver and kidneys following intra-rectal acetic acid administration, compared to the untreated controls. Argan oil administration, however, resulted in improvement of GST activity in all the tissues, with significant ($p < 0.05$) increase in GST activity observed in the kidneys, compared to the acetic acid-treated rats.

Furthermore, intra-rectal acetic acid administration led to significant ($p < 0.05$) decrease in superoxide dismutase (SOD) activity in the colon, liver and kidney, compared to the untreated controls (Table 3). However, treatment with argan oil significantly ($p < 0.05$) prevented the acetic acid-induced decrease in SOD activity in the liver and kidneys. Rats given argan oil alone showed significantly ($p < 0.05$) improved SOD activity compared to those treated with acetic acid alone.

Effect of argan oil on haematology and serum chemistry parameters

As shown in Table 4, rats treated with acetic acid alone exhibited significant ($p < 0.05$) reduction in erythrocyte (PCV, Hb and RBC), platelets and leucocyte (WBC) indices, compared to normal untreated rats. Post-treatment of

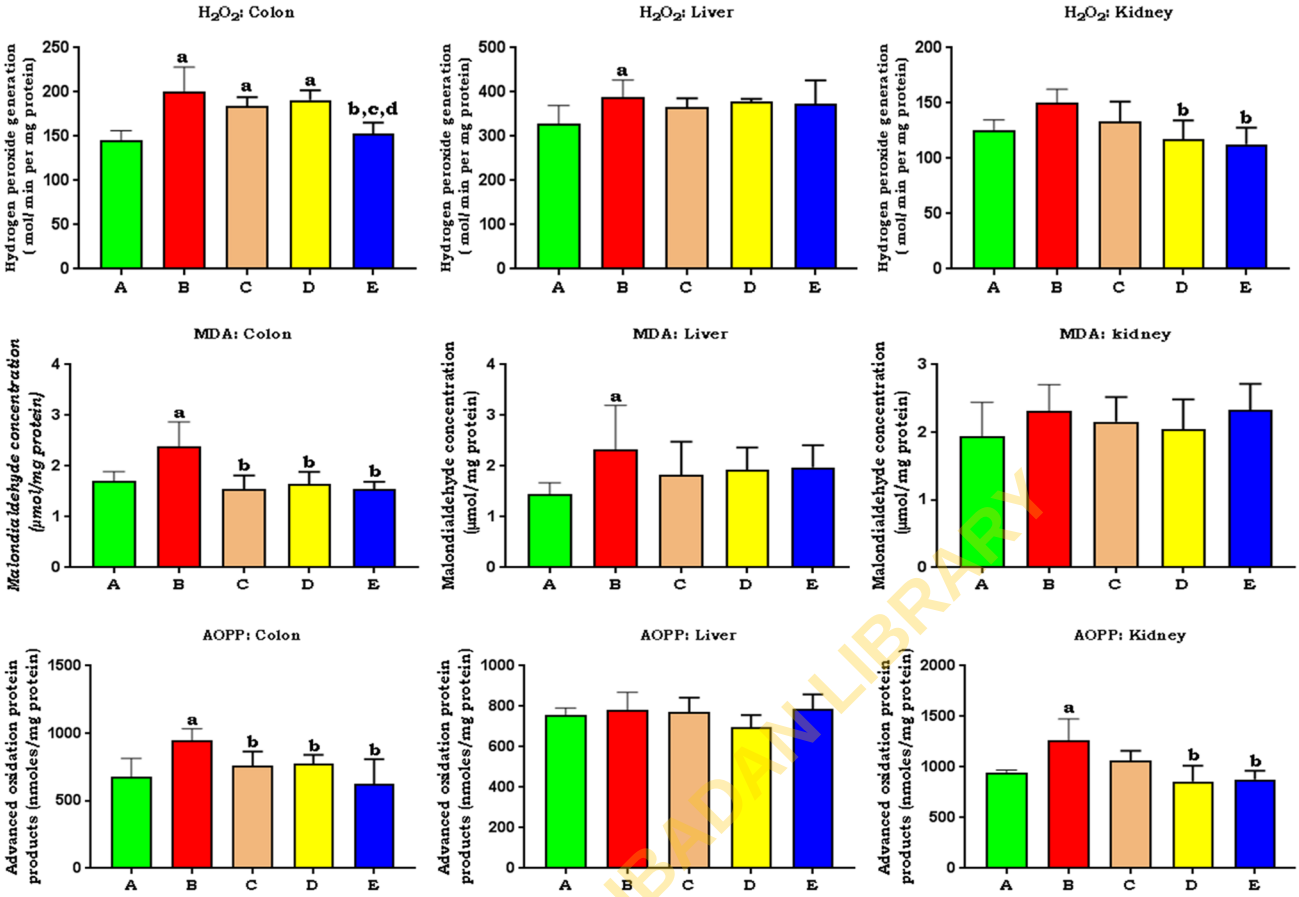

Fig. 6 Effect of Argan oil on Hydrogen peroxide (H_2O_2) concentration, Malondialdehyde (MDA) concentration and Advanced Oxidation Protein Products (AOPP) in colon, liver and kidney tissues in acetic acid-induced colitis. Bars represent mean ± standard deviation and means were compared by one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. [a]Significant ($p < 0.05$) as compared to Group A; [b]Significant ($p < 0.05$) as compared to group B; [c]Significant ($p < 0.05$) as compared to group C; [d]Significant ($p < 0.05$) as compared to group D

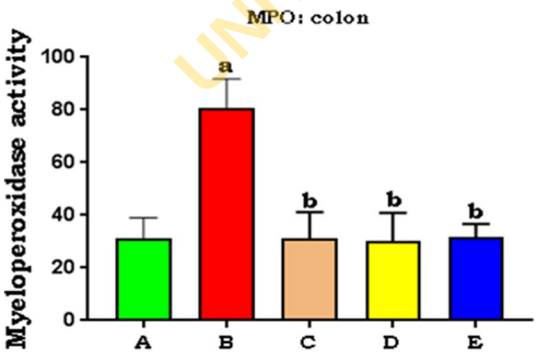

Fig. 7 Effect of Argan oil on colonic myeloperoxidase (MPO) activity in acetic acid-induced colitis. Data are presented as mean ± standard deviation and means were compared by one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. [a]Significant ($p < 0.05$) as compared to Group A; [b]Significant ($p < 0.05$) as compared to group B

rats with Argan oil after acetic acid exposure did not produce reversal of the haematological alterations induced by acetic acid as the values were still lower than those of the untreated rats. Similarly, rats pre-treated with Argan oil prior to acetic acid induction showed values that were not significantly from those of the normal untreated rats. Surprisingly, rats treated with argan oil alone also showed significantly ($p < 0.05$) lower PCV, Hb, RBC, WBC and platelet values compared to the normal untreated rats.

As presented in Table 5, acetic acid treatment resulted in significant ($p < 0.05$) increase in serum urea concentration as compared to normal untreated rats. Administration of Argan oil prior to induction of acetic acid colitis significantly ($p < 0.05$) prevented the acetic acid-induced increase in serum urea concentration when compared to the acetic acid control rats. Similarly, serum urea concentration in rats treated with Argan oil alone was significantly ($p < 0.05$) lower than that in acetic acid control rats. The only other noticeable change observed in the serum chemistry

Table 3 Effect of Argan oil on antioxidant parameters in acetic acid-induced colitis

	A	B	C	D	E
Reduced glutathione (GSH)(mol/g tissue)					
Colon	99.96 ± 6.78	88.10 ± 4.83[a]	86.64 ± 3.66[a]	87.90 ± 3.67[a]	87.99 ± 3.23[a]
Liver	84.64 ± 8.84	82.42 ± 3.18	78.61 ± 5.24	88.56 ± 6.47	89.00 ± 6.08
Kidneys	90.52 ± 4.70	88.13 ± 3.88	86.38 ± 4.18	88.72 ± 3.62	89.96 ± 4.10
Glutathione S-transferase (GST)activity (mmol CDNB-GSH complex formed/min per mg protein)					
Colon	0.08 ± 0.01	0.04 ± 0.01[a]	0.06 ± 0.02	0.05 ± 0.01	0.05 ± 0.02
Liver	0.17 ± 0.02	0.12 ± 0.03[a]	0.16 ± 0.03	0.17 ± 0.03	0.15 ± 0.02
Kidneys	0.09 ± 0.03	0.04 ± 0.01[a]	0.08 ± 0.01[b]	0.08 ± 0.02[b]	0.07 ± 0.02[b]
Superoxide Dismutase (SOD) activity (units/mg protein)					
Colon	2.32 ± 0.21	1.99 ± 0.23[a]	2.26 ± 0.29	2.15 ± 0.35	2.39 ± 0.35[b]
Liver	1.49 ± 0.21	1.06 ± 0.17[a]	1.28 ± 0.11	1.45 ± 0.28[b]	1.40 ± 0.16[b]
Kidneys	3.69 ± 0.40	1.90 ± 0.28[a]	2.96 ± 0.69[b]	3.01 ± 0.54[b]	2.99 ± 0.38[b]

Data are presented as mean ± standard deviation and means were compared by one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test

[a]Significant ($p < 0.05$) as compared to Group A

[b]Significant ($p < 0.05$) as compared to group B

[c]Significant ($p < 0.05$) as compared to group C

[d]Significant ($p < 0.05$) as compared to group D

Table 4 Effect of Argan oil on haematological parameters in acetic acid-induced colitis

Haematologicalparameters	A	B	C	D	E
PCV (%)	38.8 ± 2.78	27.75 ± 6.5[a]	27.40 ± 5.60[a]	36.00 ± 3.37	23.40 ± 4.83[a,d]
Hb (mg/dL)	12.54 ± 0.78	8.63 ± 2.23[a]	8.70 ± 1.83[a]	11.68 ± 1.20	7.38 ± 1.61[a,d]
Rbc count (x10^3cells/mm^3)	8.47 ± 1.10	4.44.5 ± 1.96[a]	4.45 ± 2.00[a]	5.72 ± 2.54	3.01 ± 1.20[a]
WBC count(× 10^3 cells/mm^3)	5.74 ± 1.39	2.37 ± 1.35[a]	2.62 ± 1.46[a]	3.83 ± 0.85	1.30 ± 0.55[a,d]
Platelets(× 10^5 cells/mm^3)	3.49 ± 0.55	1.84 ± 0.73[a]	1.95 ± 0.39[a]	2.63 ± 0.93	1.33 ± 0.30[a,d]
Neutrophils (%)	62.60 ± 5.77	63.60 ± 9.24	62.50 ± 9.68	62.60 ± 6.62	64.00 ± 4.85
Lymphocytes (%)	36.60 ± 5.77	35.60 ± 8.79	37.00 ± 9.87	36.40 ± 6.73	33.80 ± 4.97

Data are presented as mean ± standard deviation and means were compared by one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test

[a]Significant ($p < 0.05$) as compared to Group A

[b]Significant ($p < 0.05$) as compared to group B

[c]Significant ($p < 0.05$) as compared to group C

[d]Significant ($p < 0.05$) as compared to group D

was significant ($p < 0.05$) reduction in total protein levels in all the treated groups as compared to the normal untreated rats. Treatment with either acetic acid or argan oil did not produce significant changes in serum activities of hepatic enzymes (ALT, AST, ALP and GGT), conjugated or total bilirubin, as well as the concentrations of serum electrolytes, including Na^+, K^+, Cl^- and HCO_3^-.

Chemical composition of argan oil

The chromatograms obtained from the GC–MS analysis of the oil is shown in Fig. 8, while the list of compounds tentatively identified and accounting for 92.79% of the total oil composition are presented in Table 6. A total of 19 compounds were identified in the oil sample. Bioactive constituents detected in the oil include phenolics, terpenes and alcohols such as 4-Hydroxy-2-methylacetophenone (10.01%), Eugenol (0.98%) and cis-p-mentha-1(7),8-dien-2-ol (1.12%). Others include fatty acids and their derivatives including Myristic acid (4.75%), Palmitic acid methyl ester (4.96%), Methoxyacetic acid, 2-tetradecyl ester (3.45%) and Gentisic acid, tri-TMS (3.17%). The remaining chemicals include alkanes and their derivatives as well as other compounds such as phthalates.

Table 5 Effect of Argan oil on serum chemistry parameters in acetic acid-induced colitis

Parameters	A	B	C	D	E
Na^+ (mmol/L)	140.00 ± 1.00	141.67 ± 2.52	144.67 ± 1.53	142.67 ± 1.53	143.00 ± 1.00
K^+ (mmol/L)	4.13 ± 0.06	4.2 ± 0.21	4.23 ± 0.21	4.27 ± 0.06	4.07 ± 0.06
Cl^- (mmol/L)	103.33 ± 2.89	108.33 ± 2.89	111.67 ± 2.89	106.67 ± 2.89	105.00 ± 5.00
HCO_3^- (mmol/L)	22.33 ± 1.53	21.33 ± 1.53	20.33 ± 1.53	21.00 ± 1.00	22.33 ± 1.16
Urea (mg/dL)	33.33 ± 1.53	63.67 ± 4.73[a]	55.00 ± 8.71	50.33 ± 2.52[b]	47.67 ± 3.79[b]
Creatinine (mg/dL)	0.73 ± 0.06	1.03 ± 0.32	1.13 ± 0.21	1.03 ± 0.12	0.93 ± 0.06
Total protein (g/dL)	7.10 ± 0.10	6.73 ± 0.21[a]	6.67 ± 0.31[a]	6.67 ± 0.21[a]	6.57 ± 0.23[a]
Albumin (g/dL)	3.97 ± 0.06	3.67 ± 0.25	3.70 ± 0.35	3.67 ± 0.15	3.67 ± 0.31
Globulin (g/dL)	3.13 ± 0.15	3.07 ± 0.15	2.97 ± 0.12	3.00 ± 0.10	2.90 ± 0.20
Total bilirubin (mg/dL)	0.50 ± 0.20	0.80 ± 0.14	0.93 ± 0.31	0.93 ± 0.15	0.70 ± 0.14
Conjugated bilirubin(mg/dL)	0.26 ± 0.12	0.37 ± 0.12	0.43 ± 0.25	0.47 ± 0.06	0.30 ± 0.10
AST (U/L)	13.00 ± 2.00	14.5 ± 2.12	15.33 ± 2.89	15.50 ± 0.71	15.0 ± 1.41
ALT (U/L)	11.0 ± 1.00	13.00 ± 1.41	12.67 ± 3.22	13.00 ± 1.00	12.67 ± 2.52
ALP (U/L)	53.33 ± 6.66	50.50 ± 2.12	57.67 ± 8.39	57.00 ± 2.83	58.00 ± 0.00
GGT (U/L)	8.33 ± 1.53	9.50 ± 0.71	9.33 ± 3.06	9.50 ± 0.71	9.30 ± 3.06

Data are presented as mean ± standard deviation and means were compared by one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test

aSignificant ($p < 0.05$) as compared to Group A

bSignificant ($p < 0.05$) as compared to group B

cSignificant ($p < 0.05$) as compared to group C

dSignificant ($p < 0.05$) as compared to group D

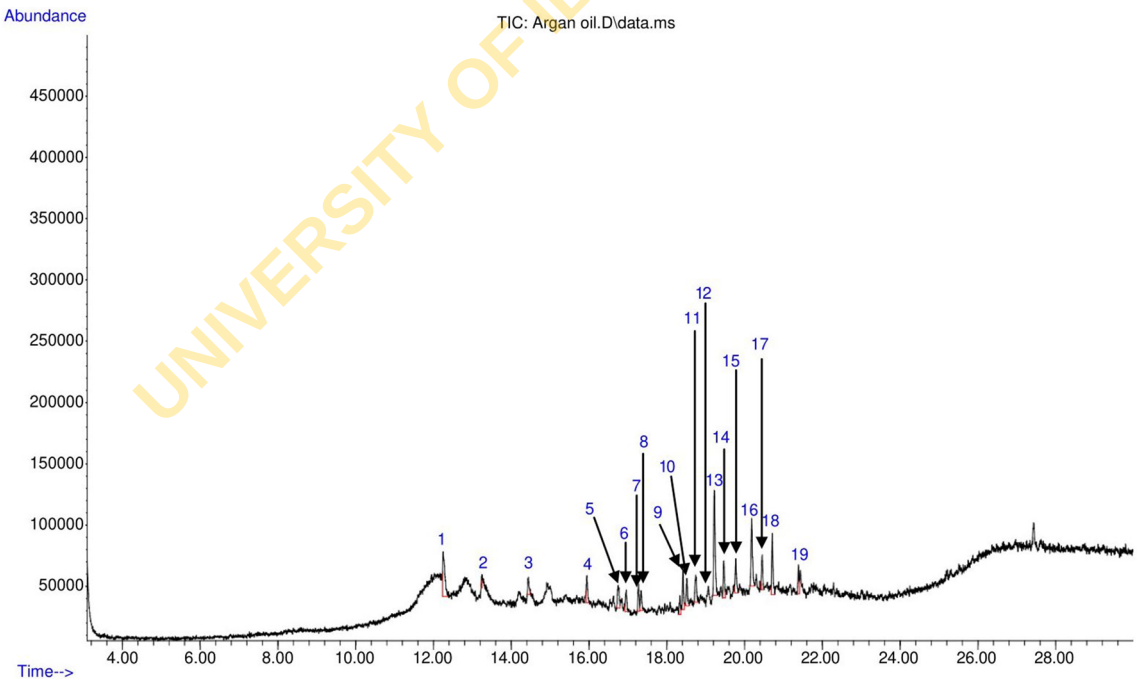

Fig. 8 Typical total ion GC–MS chromatogram of volatile constituents of argan oil. The identified compounds (represented as peaks) are indicated as numbers **(1)** 4-Hydroxy-2-methylacetophenone; **(2)** Eugenol; **(3)** Dodecamethyl Pentasiloxane; **(4)** Undecane; **(5)** (7a-Isopropenyl-4,5-dimethyloctahydroinden-4-yl)methanol; **(6)** Gentisic acid, tri-TMS; **(7)** Methoxyacetic acid, 2-tetradecyl ester; **(8)** Farnesane; **(9)** 1,2–15,16-Diepoxyhexadecane; **(10)** Isopentacosane; **(11)** 1-chloro octadecane; **(12)** Myristic acid; **(13)** Diisobutyl phthalate; **(14)** 1-chloro Heptacosane; **(15)** Palmitic acid, methyl ester; **(16)** Octyl butyl phthalate**; (17)** 1-Bromoeicosane; **(18)** i-Propyl 14-methyl-pentadecanoate; **(19)** cis-p-mentha-1(7),8-dien-2-ol

Table 6 Volatile constituents of Argan oil

s/n	Retention time (min)	Compound name/Putative identification	Molecular formula	Area (%)
1	12.248	4-Hydroxy-2-methylacetophenone	$C_9H_{10}O_2$	10.01
2	13.244	Eugenol	$C_{10}H_{12}O_2$	0.98
3	14.440	Dodecamethyl Pentasiloxane	$C_{12}H_{36}O_4Si_5$	2.34
4	15.945	Undecane	$C_{11}H_{24}$	3.49
5	16.752	(7a-Isopropenyl-4,5-dimethyloctahydroinden-4-yl)methanol	$C_{15}H_{26}O$	4.15
6	16.958	Gentisic acid, tri-TMS	$C_{16}H_{30}O_4Si_3$	3.17
7	17.272	Methoxyacetic acid, 2-tetradecyl ester	$C_{17}H_{34}O_3$	3.45
8	17.335	Farnesane	$C_{15}H_{32}$	2.99
9	18.337	1,2–15,16-Diepoxyhexadecane	$C_{16}H_{30}O_2$	2.29
10	18.417	Isopentacosane	$C_{25}H_{52}$	4.90
11	18.514	1-chloro octadecane	$C_{18}H_{37}Cl$	3.94
12	18.743	Myristic acid	$C_{14}H_{28}O_2$	4.75
13	19.218	Diisobutyl phthalate	$C_{16}H_{22}O_4$	17.12
14	19.470	1-chloro Heptacosane	$C_{27}H_{55}Cl$	5.11
15	19.773	Palmitic acid, methyl ester	C17H34O2	4.96
16	20.179	Octyl butyl phthalate	$C_{20}H_{30}O_4$	10.40
17	20.454	1-Bromoeicosane	$C_{20}H_{41}Br$	4.22
18	20.711	i-Propyl 14-methyl-pentadecanoate	$C_{19}H_{38}O_2$	3.40
19	21.381	cis-p-mentha-1(7),8-dien-2-ol	$C_{10}H_{16}O$	1.12
	Total			92.79
Class				
Carboxylic acid and esters				19.73
Alkanes and alkane derivatives				26.94
Terpenes, Phenols and Alcohols				16.26
Others				29.86

Discussion

Therapeutic strategies against ulcerative colitis (UC) include the use of anti-inflammatory drugs such as corticosteroids, although the complex nature of the pathogenesis of colitis involving reactive oxygen species, immune and genetic factors, often make the disease persistent and the treatment complicated (Ho et al. 2006; Reuter et al. 2012). Furthermore, synthetic drugs used for treatment of UC are not completely promising as they also present a variety of side effects (Bilsel et al. 2002). When present in the intestinal lumen, acetic acid has been shown to dissociate into protons which diffuses into intracellular compartments leading to acidification of epithelial cells and induction of UC via stimulation of lipid peroxidation and the cyclooxygenase pathways (Elson 1996). Hence, the selection of more comprehensive therapeutic approaches, such as medicinal plant products, that simultaneously target inflammatory and oxidant components of the disease, with fewer side effects may be of benefit in management of UC.

Intra-rectal administration of 4% acetic acid in the present study resulted in progressive decrease in body weights, most probably due to reduced food and water intake (data not shown) as also reported in previous studies (Patil et al. 2012; Kumar et al. 2014). This was accompanied by increased colon weight, decreased colon length, a corresponding increase in the colon weight/length ratio, along with local inflammation, swelling, goblet cell hyperplasia, wall thickening and shortening, all of which are characteristically observed in UC (Owusu et al. 2020). Moreover, mascroscopic examination of the colon revealed signs of colitis including severe mucosal and submucosal ulceration and necrosis, as well as reduction in stool consistency evidenced by passage of watery diarrheic faeces. However, the absolute and relative weights of the liver and kidneys were unaffected in all the different groups of rats.

In contrast to acetic acid-treated treatment, rats pretreated or post-treated with Argan oil exhibited considerable amelioration of the acetic acid-induced decrease in body weights, while the colon weight/length ratio was also considerably improved. These animals showed well-preserved colonic mucosa without evidence of erosions or blood stains, while they also passed well-formed faecal pellets. Rats treated with Argan oil had organosomatic indices that largely

resembled those of the untreated controls, indicating that the oil did not evoke negative effects on body weight and organosomatic indices. The improvement of body weight in Argan oil-treated rats may be related to a stimulation of appetite as well as inhibition of body fluid losses via bleeding or diarrhea (Hunschede et al. 2017). These findings are indicative of the therapeutic and/or prophylactic potential probably mediated by an inhibition of inflammatory events in the colon (Awaad et al. 2017).

Histopathological evaluation of colonic tissues provided corresponding evidence of the potential of Argan oil to protect the rats and reduce progression of UC. It was revealed that Argan oil treatment before or after acetic acid induction provided considerable preservation of the cytoarchitecture of the colonic mucosa against morphological alterations including necrosis, erosions ulcerations and inflammatory cell infiltration caused by acetic acid. The findings from the present study are consistent with those in prior studies utilizing the acetic acid-induced UC model (Al-Shariff et al. 2022). Interestingly, acetic acid treatment also produced congestion in central venules of the liver and some renal vessels, implying the occurrence of remote vascular disturbances such as impaired venous drainage in these organs (Yilmaz et al. 2013). Additionally, the renal tissues also showed poor architecture with mild necrosis and sclerosis of the glomeruli. Previous studies also allude that acetic acid-induced colitis is often associated with acute injury to extra-intestinal organs such as the kidneys and liver (Mohammed et al. 2020). However, Argan oil treatment resulted in marked alleviation of the morphological alterations induced by acetic acid in these tissues.

Influx of inflammatory cells into the injured colon with release of inflammatory mediators and production reactive oxygen species and oxidative stress are hallmarks of UC. In healthy rats, the tissues are normally protected by a protective antioxidant system which includes reduced glutathione (GSH), an intracellular free radical scavenger, and superoxide dismutase (SOD) as indispensable components (Dziąbowska-Grabias et al. 2021). Enzymes such as Glutathione S-transferase (GST) also play vital antioxidant roles in electrophile detoxification using GSH as electron donor (Coles and Kadlubar 2003). SOD maintains intracellular redox balance by catalyzing the reduction of noxious superoxide radicals to the less reactive hydrogen peroxide, which can then be broken down to molecular oxygen and water by the enzyme catalase (Kruidenier et al. 2003). Oxidative stress resulting from oxidant/antioxidant imbalance in favor of the oxidants has been associated with oxidative damage to tissues via processes involving lipid peroxidation and protein oxidation which have been found to be related to the development and progression of UC (Ayala et al 2014; Rana et al. 2014).

In the present study, the colonic levels of H_2O_2, MDA, a lipid peroxidation product and AOPP, product of protein oxidation, were all significantly elevated in acetic acid-induced colitis, whereas Argan oil administration before or after acetic acid induction significantly decreased MDA and AOPP levels. It was interesting to also observe evidence of oxidative stress in other remote tissues, including significantly elevated H_2O_2 and MDA levels in the liver and increased AOPP levels in the kidneys. In the kidneys, pre-treatment with Argan oil proved to be more effective in combating oxidative with a reduction in H_2O_2 and AOPP levels in contrast to post-treated rats. Rats treated with Argan oil alone showed consistently low levels of H_2O_2, MDA and AOPP in the colon, liver and kidneys. Significant inhibition of H_2O_2 generation, MDA and AOPP levels, especially in the colon, indicates profound antioxidant potential of Argan oil which was likely to be very important in the protection of colonic tissues from acetic acid-induced injury (Bakour et al. 2018). Further evidence of the anti-oxidative potential of Argan oil was observed with improvement in SOD and GST activities in the tissues and more significantly in the kidneys compared to the colitis controls which otherwise showed significant reduction in the activities of these enzymes. Treatment with argan oil did not produce significant improvement of GSH concentration in the colon, when compared with the colitis controls and this probably indicates extensive utilization of GSH due to increased demand for detoxification activities, compared to normal untreated rats (Kurutas 2016).

Several researchers have reported an increase in neutrophil infiltration of the colon with corresponding increase in myeloperoxidase activity as important hallmarks of the inflammatory response in ulcerative colitis (Cetinkaya et al. 2005). Myeloperoxidase is a glycosylated haemoprotein enzyme predominantly found in the neutrophils. Its activity is often directly proportional to the number of neutrophils in inflamed tissues and is effectively used as a quantitative index of the severity of inflammation (Hansberry et al. 2017). In our study, acetic acid administration produced significant stimulation of colonic MPO activity which was also corroborated by increased inflammatory cell infiltration. However, treatment with argan oil either alone or in combination with acetic acid resulted in dramatic reversal of the inflammatory actions of acetic acid in the colon. This result highlights the anti-inflammatory activities of components of argan oil as previously reported in its ability to attenuate inflammation in a carrageenan-induced rat model of inflammation (Menni et al. 2020).

The present findings on the therapeutic and preventive efficacy of argan oil against ulcerative colitis are supported by previous reports on the anti-colitic effects of essential oils. For instance, Rashidian et al (2016) reported that Ocimum basilicum essential oil protected against acetic

acid-induced colitis lesions via anti-inflammatory activities that caused reduction in myeloperoxidase activity. In another study, essential oils from *Bunium persicum* were found to reduce the severity of acetic acid colitis by reducing MPO activity, TNFα levels and NF-κB expression in colon tissues (Rashidian et al. 2021). Similar protection has also been obtained with ginger oil (Rashidian et al. 2014); Coriander oil (Heidari et al. 2016) and *Foeniculum vulgare* essential oil (Rezayat et al. 2018).

Our data revealed an interesting finding which suggested that treatment of rats with argan oil alone resulted in significantly lower erythrocyte and leucocyte values, compared to both control rats and rats pre-treated with Argan oil but similar to those of acetic acid-treated rats. Lower counts of blood cells in colitic rats are a usual clinical finding of colitis and have been associated with an accelerated destruction of erythrocytes via oxidative stress or loss due to haemorrhage (Kumar et al. 2014). However, the reason for significantly low erythrocyte and leucocyte values in argan oil-treated rats was unclear. Previous reports offer the suggestion that the detrimental effect of argan oil on blood cells may be related its anti-proliferative actions on blood producing cells of the bone marrow. Argan oil was found to exert inhibitory effects on the proliferation of leukaemia cell lines including JURKAT, MOLT3 and DND41 cell lines, despite their phenotypic differences (Aribi et al. 2016). This inhibitory effect of argan oil was found to involve modulation of the activity of the oncogenic Notch 1 and ERK pathways whose overactivation is known to be a key event in the pathogenesis of several solid and haematologic cancers. It thus, appears that constituents of argan oil may selectively target the haematological tissues in their anti-proliferative actions. Although these anti-proliferative effects of argan oil may be beneficial against treatment of leukemias and other haematologic cancers, it may represent an adverse effect on otherwise healthy subjects via an inhibition of molecular pathways involved in normal cell proliferation. Our results, however, indicate that this effect may only be transient as rats pre-treated with argan oil prior to acetic acid induction appeared to recover and had haematological indices closer to the values in control rats.

Argan oil (food-grade or cosmetic grade) has been reported to be rich in unsaturated fatty acids (e.g. oleic and linoleic acids) and saturated fatty acids (e.g. palmitic acid, stearic acid and myristic acid), polyphenols, sterols, tocopherols, etc. (Gharby and Charrouf 2021). The volatile constituents of argan oil are, however, composed of different chemical families including acids, alcohols, aldehydes, hydrocarbons, ketones, esters, terpenes, *N*-heterocyclic compounds and furans (El Monfalouti et al. 2013), many of which are responsible for the aroma of argan oil. In the present study, we employed untargeted qualitative GC–MS analysis of the commercial argan oil product in order to screen volatile chemical compounds present in the oil using a single sample injection into the GC column. From a total of 19 volatile compounds identified in the oil, the predominant compounds are alkane hydrocarbons and phthalates, although other important bioactive compounds with antioxidant and anti-inflammatory activities were also detected, among which are phenolics and fatty acid derivatives. Eugenol, a phenolic compound belonging to the class of phenylpropanoids has been shown to exert several pharmacological activities including antioxidant, anti-inflammatory, anticancer, analgesic, antibacterial and antifungal activities (Huang et al. 2015; Zhang et al. 2017; Barboza et al. 2018). Recently, eugenol was found to alleviate dextran sulfate sodium-induced colitis by suppressing colonic inflammation and oxidative stress (Chen et al. 2021). The oil used in this study was also found to contain palmitic acid methyl ester, a fatty acid methyl ester which reportedly possesses inhibitory activities against inflammatory cells. This palmitic acid derivative has been shown to inhibit several anti-inflammatory markers including reduction of plasma levels of TNFα and Interleukin-6, as well as reduction in expression of NF-κB in liver and lungs (Saeed et al. 2012). Furthermore, a high content of 4-Hydroxy-2-methylacetophenone, a sesquiterpene lactone, was detected in the argan oil sample. This compound was found to possess antibacterial and antifungal properties (Gómez García-Carpintero et al. 2011). One limitation to our present study is the lack of derivatization procedures that could have rendered fatty acids in the oil sample to be sufficiently volatile to be eluted. This could explain the absence of widely reported fatty acid components of argan oil such as oleic and linoleic acids.

Conclusions

This study showed the protective efficacy of argan oil against local (colonic) alterations induced by acetic acid in rats. The prophylactic and curative effects of argan oil involved its ability to decrease oxidative stress and inflammatory responses and the elevation of antioxidant capacity of the tissues. Assessment of symptoms of acetic acid induced injury including decreased body weight and organosomatic indices (colonic shortening and increased colon weight/length ratio), morphological and biochemical changes indicated that the administration of argan oil prior to acetic acid induction appeared to offer better protection than post-treatment with argan oil. Our analysis of argan oil showed the presence of some phenolics and terpenoids with antioxidant and anti-inflammatory activities which may be associated with the observed protection. Our data showed the possibility of systemic haematologic effects

which requires future clarification in assessing the overall safety of the oil for medicinal use. Overall, our findings suggest that argan oil and its components have good prospects for development as agent for the prevention and/or treatment of inflammatory intestinal disorders.

Funding No funding was received for conducting this study.

Declarations

Ethical approval This study was conducted according to guidelines outlined in the National Institute of Health publication, "Guide for the Care and Use of Laboratory Animals" (Publication No. 85–23) and also followed protocols approved by the Animal Care and Use Research Ethics Committee (ACUREC) of the University of Ibadan.

Conflict of interest Folake Olayinka Olojo has no conflict of interest. Akinleye Stephen Akinrinde has no conflict of interest. Stella Ajedawun Ogundairo has no conflict of interest. Vincetia Chinwendu Ubochi has no conflict of interest.

References

Alsharif IA, Fayed HM, Abdel-Rahman RF, Abd-Elsalam RM, Ogaly HA (2022) miconazole mitigates acetic acid-induced experimental colitis in rats: insight into inflammation, oxidative stress and Keap1/Nrf-2 signaling crosstalk. Biology 11(2):303. https://doi.org/10.3390/biology11020303

Aribi B, Zerizer S, Kabouche Z, Screpanti I, Palermo R (2016) Effect of *Argania spinosa* oil extract on proliferation and Notch 1 and ERK1/2 signaling of T-cell acute lymphoblastic leukemia cell lines. Food Agric Immunol 27(3):350–357

Awaad AS, Alafeefy AM, Alasmary FAS, el-Meligy RM, Alqasoumi SI, (2017) Anti-ulcerogenic and anti-ulcerative colitis (UC) activities of seven amines derivatives. Saudi Pharm J 25(8):1125–1129. https://doi.org/10.1016/j.jsps.2017.07.003

Ayala A, Muñoz MF, Argüelles S (2014) Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxid Med Cell Longev. https://doi.org/10.1155/2014/360438

Bakour M, Soulo N, Hammas N, Fatemi HE, Aboulghazi A, Taroq A, Abdellaoui A, Al-Waili N, Lyoussi B (2018) The antioxidant content and protective effect of Argan oil and syzygium aromaticum essential oil in hydrogen peroxide-induced biochemical and histological changes. Int J Mol Sci 19(2):610. https://doi.org/10.3390/ijms19020610

Barboza JN, Filho CMB, Silva RO, Medeiros JVR, de Sousa DP (2018) An Overview on the Anti-inflammatory potential and antioxidant profile of eugenol. Oxid Med Cell Longev. https://doi.org/10.1155/2018/3957262

Barlas AM, Kuru S, Cavusoglu T, Bag YM, Senes M, Cihan N, Celepli P, Unal Y, Hucumenoglu S (2018) Rectal application of argan oil improves healing of colorectal anastomosis in rats. Acta Cir Bras 33(7):565–576

Basholli-Salihu M et al (2017) Phytochemical composition, anti-inflammatory activity and cytotoxic effects of essential oils from three *Pinus spp*. Pharm Biol 55(1):1553–1560

Beutler EO, Duron B, Kelly M (1963) Improved method for the determination of blood glutathione. J Lab Clin Med 61:882–888

Bilsel Y, Bugra D, Yamaner S, Buluta T, Cevikbas U, Turkogluc U (2002) Could honey have a place in colitis therapy? Effects of honey, prednisolone, and disulfiram on inflammation, nitric oxide, and free radical formation. Dig Surg 19:306–312

Cagin YF, Parlakpinar H, Vardi N, Polat A, Atayan Y, Erdogan MA, Tanbek K (2016) Effects of dexpanthenol on acetic acid-induced colitis in rats. Exp Ther Med 12(5):2958–2964. https://doi.org/10.3892/etm.2016.3728

Cetinkaya A, Bulbuloglu E, Kurutas EB, Ciralik H, Kantarceken B, Buyukbese MA (2005) Beneficial effects of *N*-acetylcysteine on acetic acid-induced colitis in rats. Tohoku J Exp Med 206(2):131–139. https://doi.org/10.1620/tjem.206.131

Cetinkaya, et al (2006) Effects of L-carnitine on oxidant/antioxidant status in acetic acid-induced colitis. Dig Dis Sci 51(3):488–494

Chen S, Wu X, Tang S, Yin J, Song Z, He X, Yin Y (2021) Eugenol alleviates dextran sulfate sodium-induced colitis independent of intestinal microbiota in mice. J Agric Food Chem 69(36):10506–10514. https://doi.org/10.1021/acs.jafc.1c00917

Coles BF, Kadlubar FF (2003) Detoxification of electrophilic compounds by glutathione S-transferase catalysis: determinants of individual response to chemical carcinogens and chemotherapeutic drugs? BioFactors 17(1–4):115–130. https://doi.org/10.1002/biof.5520170112

Drury RA, Wallington EA, Cancerson R (1976) Carlton's histopathological techniques, 4th edn. Oxford UniversityPress, London, New York

Dziąbowska-Grabias K, Sztanke M, Zając P, Celejewski M, Kurek K, Szkutnicki S, Korga P, Bulikowski W, Sztanke K (2021) Antioxidant therapy in inflammatory bowel diseases. Antioxidants 10:412

El Monfalouti H, Charrouf Z, Giordano M, Guillaume D, Kartah B, Harhar H et al (2013) Volatile compound formation during argan kernel roasting. Nat Prod Comm 8:33–36. https://doi.org/10.1177/1934578X1300800108

Elson CO (1996) The basis of current and future therapy for inflammatory bowel disease. Am J Med 100(6):656–662

Gasche C, Lomer MCE, Cavill I, Weiss G (2004) Iron, anaemia, and inflammatory bowel diseases. Gut 53:1190–1197. https://doi.org/10.1136/gut.2003.035758

Gharby S, Charrouf Z (2021) Argan oil: chemical composition, extraction process, and quality control. Front Nutr. https://doi.org/10.3389/fnut.2021.804587

Gómez García-Carpintero E, Sánchez-Palomo E, González-Viñas MA (2011) Aroma characterization of red wines from cv. Bobal grape variety grown in La Mancha region. Food Res Int 44(1):61–70

Habig WH, Pabst MJ, Jakoby WB (1974) Glutathione-S-transferases. The first enzymatic step in mercapturic acid formation. J Biol Chem 25:7130–7139

Hanana M, Mezghenni H, Ben Ayed R, Ben Dhiab A, Jarradi S, Jamoussi B, Hamrouni L (2018) Nutraceutical potentialities of Tunisian Argan oil based on its physicochemical properties and fatty acid content as assessed through Bayesian network analyses. Lipids Health Dis 17(1):138. https://doi.org/10.1186/s12944-018-0782-9

Hansberry DR, Shah K, Agarwal P, Agarwal N (2017) Fecal myeloperoxidase as a biomarker for inflammatory bowel disease. Cureus 9:e1004

Heidari B, Sajjadi SE, Minaiyan M (2016) Effect of *Coriandrum sativum* hydroalcoholic extract and its essential oil on acetic acid- induced acute colitis in rats. Avicenna J Phytomed 6(2):205–214

Ho G, Chiam P, Drummond H, Loane J, Arnott R, Satsangi J (2006) The efficacy of corticosteroid therapy in inflammatory bowel disease: Analysis of a 5-year UK inception cohort. Aliment Pharmacol Ther 24:319–330

Huang X, Liu Y, Lu Y, Ma C (2015) Anti-inflammatory effects of eugenol on lipopolysaccharide-induced inflammatory reaction in acute lung injury via regulating inflammation and redox status. Int Immunopharmacol 26(1):265–271

Hunschede S, Kubant R, Akilen R, Thomas S, Anderson GH (2017) Decreased appetite after high-intensity exercise correlates with increased plasma interleukin-6 in normal-weight and overweight/

obese boys. Curr Dev Nutr 1(3):e000398. https://doi.org/10.3945/cdn.116.000398
Imagawa M (1999) Extra-intestinal complications of ulcerative colitis: hematologic complication. Nihon Rinsho 57(11):2556–2561
Kayali R, Cakatay U, Akcay T et al (2006) Effect of alpha-lipoic acid supplementation on markers of protein oxidation in post-mitotic tissues of ageing rat. Cell Biochem Funct 24:79–85
Ke F, Yadav PK, Ju LZ (2012) Herbal medicine in the treatment of ulcerative colitis. Saudi J Gastroenterol 18(1):3–10. https://doi.org/10.4103/1319-3767.91726
Kruidenier L, Kuiper I, Van Duijn W, Mieremet-Ooms MA, van Hogezand RA, Lamers CB et al (2003) Imbalanced secondary mucosal antioxidant response in inflammatory bowel disease. J Pathol 201:17–27
Kumar VS, Rajmane AR, Adil M, Kandhare AD, Ghosh P, Bodhankar SL (2014) Naringin ameliorates acetic acid induced colitis through modulation of endogenous oxido-nitrosative balance and DNA damage in rats. J Biomed Res 28(2):132–145. https://doi.org/10.7555/JBR.27.20120082
Kurutas EB (2016) The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: current state. Nutr J 15(1):71. https://doi.org/10.1186/s12937-016-0186-5
Lapidus A, Bångstad M, Aström M, Muhrbeck O (1999) The prevalence of gallstone disease in a defined cohort of patients with Crohn's disease. Am J Gastroenterol 94:1261–1266
Lee YM, Kaplan MM (1995) Primary sclerosing cholangitis. N Engl J Med 332:924–933
Levine JS, Burakoff R (2011) Extraintestinal manifestations of inflammatory bowel disease. Gastroenterol Hepatol 7(4):235–241
Menni HB, Belarbi M, Menni DB, Bendiab H, Kherraf Y, Ksouri R, Djebli N, Visioli F (2020) Anti-inflammatory activity of argan oil and its minor components. Int J Food Sci Nutr 71(3):307–314. https://doi.org/10.1080/09637486.2019.1650005
Misra HP, Fridovich I (1972) The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem 247(10):3170–3175. https://doi.org/10.1016/S0021-9258(19)45228-9
Mohamed DA, Ahmed SM, Kamal MM (2020) The role of retinoids in acute renal damage associated with acetic acid induced ulcerative colitis in adult male rats: histological and biochemical study. J Med Histol 3(1):44–58
Mohamed NI, El-Kashef DH, Suddek GM (2021) Flavocoxid halts both intestinal and extraintestinal alterations in acetic acid-induced colitis in rats. Environ Sci Pollut Res. https://doi.org/10.1007/s11356-021-16092-7
Monfalouti HE, Guillaume D, Denhez C, Charrouf Z (2010) Therapeutic potential of argan oil: a review. J Pharm Pharmacol 62(12):1669–1675. https://doi.org/10.1111/j.2042-7158.2010.01190.x
Owusu G, Obiri DD, Ainooson GK, Osafo N, Antwi AO, Duduyemi BM, Ansah C (2020) Acetic acid-induced ulcerative colitis in sprague dawley rats is suppressed by hydroethanolic extract of *Cordia vignei* leaves through reduced serum levels of TNF-α and IL-6. Int J Chronic Dis. https://doi.org/10.1155/2020/8785497
Patil MVK, Kandhare AD, Bhise SD (2012) Anti-inflammatory effect of *Daucus carota* root on experimental colitis in rats. Int J Pharm Pharm Sci 4:337–343
Podolsky DK (2002) Inflammatory bowel disease. N Engl J Med 347:417–429
Qin HY, Wu JC, Tong XD, Sung JJ, Xu HX, Bian ZX (2011) Systematic review of animal models of post-infectious/post-inflammatory irritable bowel syndrome. J Gastroenterol 46:164–174
Rana SV, Sharma S, Prasad KK, Sinha SK, Singh K (2014) Role of oxidative stress & antioxidant defence in ulcerative colitis patients from north India. Indian J Med Res 139(4):568–571
Rashidian A, Mehrzadi S, Ghannadi AR, Mahzooni P, Sadr S, Minaiyan M (2014) Protective effect of ginger volatile oil against acetic acid-induced colitis in rats: a light microscopic evaluation. J Integr Med 12(2):115–120
Rashidian A, Roohi P, Mehrzadi S, Ghannadi AR, Minaiyan M (2016) Protective effect of *Ocimum basilicum* essential oil against acetic acid-induced colitis in rats. J Evid Based Comp Alt Med 21(4):NP36–NP42. https://doi.org/10.1177/2156587215616550
Rashidian A, Akbarzadeh D, Asgarpanah J, Dehpour A (2021) *Bunium persicum* essential oil reduced acetic acid-induced rat colitis through suppression of NF-κB pathway. Avicenna J Phytomed 11(5):505–514. https://doi.org/10.22038/AJP.2021.18037
Reuter KC, Grunwitz CR, Kaminski BM, Steinhilber D, Heinfried H, Radeke HH et al (2012) Selective glucocorticoid receptor agonists for treatment of inflammatory bowel disease-studies in mice with acute TNBS colitis. J Pharmacol Exp Ther 341:68–80
Rezayat SM, Dehpour A-R, Motamed SM, Yazdanparast M, Chamanara M, Sahebgharani M, Rashidian A (2018) *Foeniculum vulgare* essential oil ameliorates acetic acid-induced colitis - in rats through the inhibition of NF kB pathway. Inflammopharmacology 26:851–859
Saeed NM, El-Demerdash E, Abdel-Rahman HM, Algandaby MM, Al-Abbasi FA, Abdel-Naim AB (2012) Anti-inflammatory activity of methyl palmitate and ethyl palmitate in different experimental rat models. Toxicol Appl Pharmacol 264(1):84–93. https://doi.org/10.1016/j.taap.2012.07.020
Shaaban HA, El-Ghorab AH, Shibamoto T (2012) Bioactivity of essential oils and their volatile aroma components. J Essent Oil Res 24(2):203–212
Varshney R, Kale RK (1990) Effect of calmodulin antagonists on radiation-induced lipid peroxidation in microsomes. Int J Radiat Biol 58:733–743. https://doi.org/10.1080/09553009014552121
Wester AL, Vatn MH, Fausa O (2001) Secondary amyloidosis in inflammatory bowel disease: a study of 18 patients admitted to Rikshospitalet University Hospital, Oslo, from 1962 to 1998. Inflamm Bowel Dis 7:295–300
Wolff SF (1994) Ferrous ion oxidation in the presence of ferric ion indicator xylenol orange for measurement of hydrogen peroxides. Methods Enzymol 233(2):182–189. https://doi.org/10.1016/0003-2697(92)90122-n
Xia Y, Zweier JL (1997) Measurement of myeloperoxidase in leukocyte-containing tissues. Anal Biochem 245:93–96
Yadav PK, Liu Z (2009) Current strategies for the treatment of ulcerative colitis. Recent Pat Inflamm Allergy Drug Discov 3:65–72
Yılmaz B, Köklü S, Bayraktar Y (2013) Ulcerative colitis presenting with Budd-Chiari syndrome. J Crohns Colitis 7(2):e74–e75. https://doi.org/10.1016/j.crohns.2012.07.005
Zhang LL, Zhang LF, Xu JG, Hu QP (2017) Comparison study on antioxidant, DNA damage protective and antibacterial activities of eugenol and isoeugenol against several foodborne pathogens. Food Nutr Res 61(1):1353356