Egypt. J. Chem. **Vol. 65,** No. 10 pp. 57 - 69 (2022)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

ULCERATIVE COLITIS, IMMUNE SYSTEM AND IRON STATUS INTER-RELATIONSHIP AND THE PROTECTIVE ROLE OF DATE AND GRAPE SEED EXTRACTS IN RAT MODEL

Sahar Y. Al-Okbi[1*], Enas S.K. Al-Siedy[1], Rasha S. Mohamed[1], Hoda B. Mabrok[1], Fathy M. Mehaya[2]

[1]*Nutrition and Food Sciences Department, National Research Centre, Cairo, Egypt*

[2]*Food Technology Department, National Research Centre, Cairo, Egypt.*

Abstract

Ulcerative colitis (UC) is an inflammatory bowel disease with un-elucidated pathogenesis. Thorough investigation of immune system and iron status is decisive in UC development. Safe nutraceuticals for prevention of UC are crucial due to unwanted side effects of UC remedies. The research aimed at studying the changes in immunity and iron status in UC and the protective role of date palm (DP) and grape (G) seed extracts as nutraceuticals. DP and G seed extracts were tested in rats with induced UC. Biochemical and molecular nutrition analysis were applied on blood and colon along with colon histopathology and disease index. Phenolic compounds were analyzed in the ethanol extracts. Results showed that the major phenolic compounds in date and grape seeds were chlorogenic and catechin; respectively. Iron deficiency anemia with elevated colon oxidative stress and inflammatory biomarkers related to immune system were noticed in UC control compared to normal. Both treatments showed variable improvements in the aforementioned changes. Disease index demonstrated date seed extracts to be more efficient than grape. UC histopathological alterations were slightly improved on both treatments. In conclusion; iron deficiency anemia might be interrelated to elevated cytokines and oxidative stress in UC. Both treatments showed variable improvements in UC.

Keywords: Ulcerative colitis; immunity; iron status; inflammation; oxidative stress; nuclear factor kappa light expression of inducible nitric oxide synthase; expression of cyclooxygenase-2; expression of interleukin-1β.

Introduction

Ulcerative colitis (UC) is one of the inflammatory bowel diseases and it is a chronic disease of the gastrointestinal tract. It is characterized by inflammation, mucosal tissue damage and ulcers of the colon and rectum. Weight loss, fever and anemia may occur in UC [1].The pathogenesis of the disease might be ascribed as an autoimmune mechanism however this was not fully elucidated. Proposed theories involve immune system dysfunction, alteration in microflora, genetic or environmental factors [2].

Immune system was claimed to have a vital role in the pathogenesis of UC. Understanding the meticulous job of immune system in the onset and progression of UC not only enables scientists to understand the mechanism underlying such diseases but also to find out new efficient remedies [3]. The altered immune function was reported to be represented by inflammatory cytokines and free radicals. However, an accumulating evidence of adaptive responses of the immune system to chronic diseases was another assumption. Allostasis (The process of preserving homeostasis through the adaptive change of body internal environment to meet perceived and anticipated demands) involvement rather than inflammation was claimed to reflect greater function significance of immune system [4].

Non-specific anemia is usually observed in ulcerative colitis that could have negative impact on the quality of life and survival and requires therapeutic intervention. So, ferrokinetic state must be studied well and correlated to the clinical pathway in such disease. The involvement of immune system in producing anemia might be related to both cytokines and acute phase protein [5]. On the other hand, there is a recent interest regarding the role of iron in immunity [6]. Immune function was reported to be impaired during iron-deficiency [7]. Understanding the cross-talk between iron homeostasis and immune system is crucial in ulcerative colitis. The important question is that how disturbance in iron metabolism can affect

*Corresponding author e-mail S_Y_alokbi@hotmail.com.; (Sahar Y. Al-Okbi).

Received Date: 15 October 2021; Revise Date: 10 November 2021; Accept Date: 14 November 2021.

DOI: 10.21608/EJCHEM.2021.101187.4702

©2022 National Information and Documentation Center (NIDOC)

immunity and how alteration in immune system could lead to iron imbalance [8].

So far, there is no efficient therapy for eradication of UC. The side effect of the drugs used in such disease represented by corticosteroids, sulfasalazine, mesalazine and azathioprine [9,10] aroused the importance of searching more safe natural agents for either protection or treatment. Functional food ingredients (Nutraceuticals) of anti-inflammatory, antioxidant and immune-modulatory effect could either protect from the incidence of the disease or prevent its progression. Lipophilic active constituents such as polyunsaturated fatty acids, carotenoids, vitamin E, and sterols and hydrophilic bioactive content like phenolic compounds from plant food extracts could provide immune-modulating, anti-inflammatory and antioxidant effects thereby could afford a therapeutic effect in UC. Grape and date palm seeds bioactive constituents are example of such nutraceuticals that may have a role in alleviating UC pathologic changes.

The Main goal of the present research is to study the changes in immunity related to inflammation and oxidative stress as well as iron status in UC and to investigate the nutraceuticals protective effect represented by date palm and grape seed extracts in rat model of UC.

Experimental

Materials

El Barhy date from the Egyptian variety (*Foenix dactylifera*, family Arecaceae) and an Egyptian grape (*Vitis vinifera*, family Vitaceae) were purchased from local market, Egypt. Date seeds and grape seeds were separated from the fruits, washed by distilled water and dried individually in a hot air oven at 40ºC. The dried seeds were crushed.

Dextran Sulphate sodium (Mw: 500 000) was purchased from Bio Basic Canada, INC for induction of ulcerative colitis in rats. All other used chemicals during the study were of high grade.

Animals

Male Sprague-Dawley rats of body weight ranging from 100 to 130 g were purchased from the Animal House of National Research Centre. Rats were kept individually in stainless steel cages at ambient temperature 25°C ± 2, with 12h light/dark cycle. Food and water were supplied *ad-libitum*. Handling and care of animals was carried out according to the Medical Research Ethics Committee, National Research Centre, Cairo, Egypt (Registration No. 19175) and followed the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985). Rats were acclimatized on balanced diet for a week before starting the experiment.

Preparation of the extracts

Known weight of crushed dried grape seeds and date seeds were successively extracted separately by petroleum ether followed by absolute ethanol using continuous extraction apparatus (soxhlet). The solvents were evaporated from each extract at temperature not exceeding 40ºC under reduced pressure.

Preparation of nutraceuticals

1)Nutraceutical 1(N1) was prepared as emulsion by mixing grape seed petroleum ether extract (219 mg) and 381mg alcohol extract (1:1.74 w/w) with the addition of 22 mg from tween 80 as 10% from the weight of the petroleum ether extract and completed by distilled water to 12 ml.

2) Nutraceutical 2 (N2) was prepared by mixing date seed petroleum ether extract (105.3 mg) and 495 mg ethanol extract (1:4.7 w/w) with the addition of 10.5 mg from tween 80 as 10% from the weight of the petroleum ether extract and completed by distilled water to 12 ml.

The ratio of petroleum ether extract to alcohol extract was selected based on the presence of such ratio in nature in the studied seeds as shown from the extraction yield of both extracts.

Gas chromatography–mass spectrometry analysis (GC-MS) for fatty acids methyl esters (FAME) of the petroleum ether extracts of date and grape seeds

The GC-MS system (Agilent Technologies) was equipped with gas chromatograph (7890B) and mass spectrometer detector (5977A) at Central Laboratories Network, National Research Centre, Cairo, Egypt. The GC was equipped with HP-5MS column (30 m x 0.25 mm internal diameter and 0.25 μm film thickness). Analyses were carried out using helium as the carrier gas at a flow rate of 1.0 ml/min, injection volume of 1 µl and the following temperature program: 50 °C for 1 min; rising at 20 °C /min to 200 °C and held for 5 min; rising at 3 °C/min to 230 °C and held for 23 min. The injector and detector were held at 250 °C. Mass spectra were obtained by electron ionization (EI) at 70 eV and using a spectral range of m/z 20-550 and solvent delay 1.8 min. Identification of different constituents was determined by comparing the spectrum fragmentation pattern with those stored in Wiley and NIST Mass Spectral Library data.

Determination of phenolic acid and flavonoids in ethanol extracts of date and grape seed

Phenolic acids and flavonoids were determined in date and grape seed ethanol extracts according to the previous method [11]. One gram of each sample was mixed with 20 ml of 2 M sodium hydroxide in stopper flasks and flushed with N2 then the stoppers were placed followed by shaking for 4 hours. The pH was adjusted to 2 with 6M hydrochloric acid. Centrifugation was carried out at 5,000 rpm for 10 minutes. The supernatant was collected. The phenolic compounds were extracted twice by ethyl ether and ethyl acetate in the ratio of 1:1. The organic phase was separated and the solvent was evaporated at 45°C and the residues were re-dissolved in 2 ml methanol and analyzed by High Performance Liquid Chromatography (HPLC). Agilent Technologies 1100 series liquid chromatography equipped with degasser, quaternary pump, autosampler, diode-array, and fluorescence detectors were used. The analytical column was Eclipse XDB-C18 (150 X 4.6 µm; 5 µm) with a C18 guard column (Phenomenex, Torrance, CA). Phenolic acids and flavonoids were identified and calculated by comparison with standards.

Preparation of a balanced diet for feeding the rats

A balanced diet was prepared to feed rats all over the experiment. The diet consisted of 12% casein, 10% sunflower oil, 68.5% corn starch, 5% wheat bran, 3.5% salt mixture and 1% vitamin mixture.

Design of animal experiment:

Rats were divided into 4 groups, each of eight rats. The first group served as normal control (NC), the second was run as control with ulcerative colitis (UCC). The third and fourth groups were the test groups. Rats of all the groups were fed on balanced diet throughout the experiment. Rats of the test groups 3 and 4 were given daily oral doses of N1 and N2; respectively, as 250 mg/kg rat body weight for 4 weeks. At the 21th day of the experiment; drinking water was exchanged by water containing 5% dextran sodium sulphate for rats of group 2, 3 and 4 for one week to induce UC [12], then normal drinking water was given for one day. Rats were fasted and at the next day blood samples were drawn from anaesthetized rats in heparinized test tubes for determination of hemoglobin. Plasma was separated by centrifugation at 3000 rpm for further biochemical analysis. Rats were dissected; the colon was excised and the distal 10 cm were opened longitudinally. Colon was washed by saline and examined macroscopically. A part of colon was kept in 10% formalin for histopathological examination. Colon tissues were further processed for pathological examination using the automatic tissue processor and sectioned at 5 µm thickness by using the rotary microtome. Sections were stained with hematoxylin and eosin (H& E) [13] for microscopical examination. The sections were examined using Nikon research microscope. A second part of colon was used for biochemical analysis of the parameters determined by the enzyme linked immune-sorbent assay (ELIZA). A third part of colon was extracted by phosphate buffer for assessing reduced glutathione (GSH), nitric oxide (NO) and malondialdehyde (MDA). An extra part of colon was used for RNA extraction for determination of the expression of interleukin-1β (IL-1β), nuclear factor kappa light chain enhancer of activated B cells (NF-kB), inducible nitric oxide synthase, (iNOS) and cyclooxygenase-2 (COX2) by real time polymerase chain reaction (RT-PCR). During the experiment; body weight and food intake were monitored twice weekly. At the end of the experiment body weight gain, total food intake and food efficiency ratio (body weight gain/total food intake) were calculated.

Biochemical analysis of plasma:

Plasma alkaline phosphatase (ALP), iron and total iron binding capacity (TIBC) were assessed according to previous methods [14-16]. Transferrin saturation percentage was calculated using the following formula: (Plasma iron/ TIBC) X 100. Erythroferrone, soluble transferrin receptor (sTfR), interferon Gamma (IFNγ) were determined adopting ELISA Kits (Elabscience Biotechnology Inc. USA)

Biochemical analysis of colon

Colon Interleukin-6 (IL-6) was assessed using ELISA Kit (Elabscience Biotechnology Inc. USA). GSH was analyzed according to the previous methods [17], NO and MDA were assessed adopting the previously reported methods [18, 19].

Gene expression of selected gene in ulcerative colitis experiment analyzed by RT-PCR

Total RNA was isolated from colon tissue with PureLink® RNA Mini Kit (ambion® Life technologies™) according to the manufacturer's instructions. RNA concentrations were measured with a NanoDrop spectrophotometer and purity of the extracted RNA was assessed by the A_{260nm}/A_{280nm} ratio. The cDNA was synthesized from 2.0 µg of total RNA in 20µl reaction with RevertAid first strand cDNA synthesis kit (Thermo Fisher® invitrogen™) according to the manufacturer's instructions.

Real-time PCR was performed with a Rotor-Gene® MDx instrument. The RT-PCR reaction mixture (20 µl) contained 1 µl template cDNA, 1× the EvaGreen® PCR master mix (HOT FIREPol® EvaGreen® qPCR Mix Plus, Solis BioDyne™) and 0.2 µM of the primer pairs. Primers pairs sequence used for iNOS, COX-2, IL-1β and NF-kB gene expression analysis were adapted from the literature [20-22], primers sequence were presented in Table (1). PCR reactions were performed using the following protocol: 95°C for 15

min, 45 cycles of 15 seconds at 95°C, 60 seconds at 60°C, 20 seconds at 72°C, melting curve program (60-95°C). PCR water was used instead of cDNA templates as a negative control. The relative expression of the target genes was calculated using $2^{-\Delta\Delta CT}$ method [23]; the target gene expression was normalized to the expression of the house-keeping gene GAPDH.

Assessment of the disease index

Disease index was assessed through macroscopic examination of colon, feces consistency, degree of blood in feces and percentage reduction of body weight gain according to previous studies [24-26] with modifications to fit the present study.

Macroscopic evaluation of colon by scores:
0=no damage, 1=mucosal erythema, 2=mild mucosal edema, slight bleeding or erosion, 3=moderate edema, bleeding, erosion, ulcers, 4= severe edema, bleeding, erosion, ulcers

Feces consistency by scores:
0=Normal, 1=Soft (wet), 2= Very soft (semiliquid), 3= Watery diarrhea

Degree of bloody feces by scores:
0=-ve, 1=red, 2=Dark red, 3=black

Weight gain loss by scores:
0= -ve, 1=1-9% loss in body weight gain, 2=10-19% loss in body weight gain, 3= > 20% loss in body weight gain

Statistical analysis

Data were analyzed by one-way ANOVA followed by the Tukey′s multiple comparison test using the SPSS statistical program. Differences were considered significant at $p< 0.05$.

Results and Discussion

Ulcerative colitis is an inflammatory disease affecting the mucosal layer of the distal colon and rectum. The present study dealt with the changes in iron status and its relation to the immune system including inflammatory cytokines and oxidative stress in UC. ALP was determined as a biomarker of intestinal damage in UC. Pro-inflammatory cytokines like IL-6, IL-1β are involved in the immuno-pathogenesis of UC [27]. iNOS, COX2 and NF-kB are among the inflammatory biomarkers affected during UC. The protective role of anti-inflammatory, antioxidants and immuno-modulatory nutraceuticals towards UC were studied in the current work to be an alternative to the drugs of unwanted side effects that used in such disease.

Dextran sulphate sodium (DSS) was used to induce UC in rats in the present study. DSS rat model is considered the closest model that mimics human ulcerative colitis. El-Mahdy et al. [28] treated rats with 5% DSS for 5 days to induce UC while 5% DSS (36–50 kDa; MP Biomedicals) in fresh drinking water was used for 7 days to induce UC in Qiu et al. and Martin et al. studies [12,29] .

Nutritional parameters shown in Table 2, demonstrated significant reduction in body weight gain in the UCC group compared to NC group. This result is similar to what reported previously [1] which might be related to diarrhea showed during evaluation of the disease index and anorexia reflected in the significant reduced food intake in the present study and as reported previously [26]. The treated groups showed improvement in body weight gains compared to the UCC group insignificantly, but concomitantly they matches that of the NC group. Total food intake was significantly increased on administration of grape seed extract compared to the UCC group and matched NC group. Treatment with date extract showed only insignificant increase in total food intake compared to the UCC group. The improvements in the nutritional parameters of the test groups might point to the beneficial effects of both extracts towards UC.

Table 1. Primers used for real-time PCR amplifications

Target genes	Sequences
iNOS	FW (5′- ACCTTCCGGGCAGCCTGTGA -3′) RW(5′- CAAGGAGGGTGGTGCGGCTG-3′)
IL-1β	FW (5′-CAC CTT CTT TTC CTT CAT CTT TG-3′) RW(5′- GTC GTT GCT TGT CTC TCC TTG TA-3′)
COX2	FW (5′-TGGTGCCGGGTCTGATGATG-3′) RW(5′- GCAATGCGGTTCTGATACTG-3′)
NF-*k*B	FW (5′-CAGCCTGGTGGGCAAGCACT-3′) RW(5′- TTCTCACGATCTTCAGGTTGGACGCGTTG-3′)
GAPDH	FW (5′- GTATTGGGCGCCTGGTCACC -3′) RW(5′- CGCTCCTGGAAGATGGTGATGG -3′)

NF-*k*B: Nuclear factor kappa light chain enhancer of activated B cells, iNOS: Inducible nitric oxide synthase,, COX2: Cyclooxygenase-2, IL-1β: Interleukin-1β, GAPDH: Glyceraldehyde-3-phosphate dehydrogenase.

Table 2. The nutritional parameters of the different experimental groups

Groups	Initial body weight (g)	Final body weight (g)	Body weight gain (g)	Total food intake (g)	Food efficiency ratio
NC	$132.14^{a}\pm4.18$	$215.57^{a}\pm9.12$	$83.43^{b}\pm6.08$	$419.86^{b}\pm17.83$	$0.20^{a}\pm0.01$
UCC	$132.29^{a}\pm4.38$	$192.71^{a}\pm7.39$	$60.43^{a}\pm6.64$	$351.57^{a}\pm13.71$	$0.17^{a}\pm0.01$
Date seed	$132.29^{a}\pm3.26$	$207.71^{a}\pm6.99$	$75.43^{ab}\pm5.31$	$379.57^{a}\pm13.36$	$0.20^{a}\pm0.01$
Grape seed	$132.14^{a}\pm3.33$	$209.43^{a}\pm7.61$	$77.29^{ab}\pm6.37$	$432.00^{b}\pm8.60$	$0.18^{a}\pm0.01$

NC: Normal control, UCC; Ulcerative colitis control, Date seed: Group treated with date seed extracts, Grape seed: Group treated with grape seed extracts.

In each column same letters mean non-significant difference while different letters mean significant difference at $p< 0.05$. Data are expressed as mean values ± standard error.

Hemoglobin concentration, plasma iron and %transferrin saturation showed significant reduction with concomitant increase in TIBC, sTfR and erythroferrone (table 3) in the UCC group compared to the NC group denoting iron deficiency anemia (IDA) as reported previously [30]. High erythroferrone level pointed to iron deficiency anemia according to previous study [31]. When IDA occurs, erythroferrone, a hormone produced by bone marrow erythroblasts, increased and acts on hepatocytes to suppress hepcidin production and thereby increases dietary iron absorption and mobilization from stores [32,33]. It was reported that sTfR were significantly higher than normal in children with IDA [34] and the increase is proportional to tissue iron deficit. The sTfR are sensitive, early and highly quantitative marker of iron depletion. The sTfR is not an acute phase reactant and remains normal in patients with chronic inflammatory diseases unless there was an existence of IDA [35]. The sTfR is efficient in diagnosis of iron deficiency anemia especially during inflammation. The sTfR level increased if there is increased iron demand due to iron deficiency, increased erythropoiesis like hemolysis, or dyserythropoiesis like megaloplastic anemia regardless of other coexistent state. So, it can be used to demonstrate iron deficiency in patients that have also acute phase response and it can distinguish iron deficiency from anemia of chronic diseases. It is worthy to mention that patients with acute phase response have reduced plasma iron and transferrin with elevation of ferritin therefore these usual indicators are unreliable [36, 37]. In the UC patients that suffer chronic inflammation, ferritin is not a good marker for IDA because it is an acute phase reactant that increases with inflammation so it may give bias result. On the reverse, elevated erythroferrone and sTfR are good indicators of IDA. Both treatments in the present study showed significant improvements in the aforementioned parameters related to iron status denoting reduction in IDA.

In colon, GSH (Table 4) was significantly reduced along with significant increase in both MDA and NO pointing to elevated oxidative stress and initiation of inflammation which was supported by elevated IL-6, the inflammatory cytokine, (table 5) in the UCC group compared to the NC group. GSH, MDA, NO and IL-6 showed significant improvements in rats treated with either date or grape seed extract pointing to their capability to regulate immune function by reducing oxidative stress and inflammation. IFN gamma did not show any significant changes among the studied groups (Table 5). ALP (Table 4) was significantly increased in the UCC group compared to the NC group denoting some sorts of damage in the colon [38]. The test groups showed improvement in ALP reflecting a therapeutic effect through reducing colon damage.

NF-kB gene expression was significantly increased in the colon of the UCC group compared to the NC group (table, 6). The mRNA expressions of iNOS, COX2, and IL-1β were significantly up-regulated in the UCC group in comparison to the NC group. Expression of iNOS can be induced by cytokines and other agents. This expression leads to continual production of NO until the enzyme is degraded which is confirmed by the result of the present research that showed significant elevation in both iNOS and NO in the UCC group. The role of iNOS in immune system is to combat the invading microorganisms or to have antitumor action however it may have detrimental consequences and seem to be involved in the pathophysiology of chronic diseases including UC [39]. NF-kB is a protein complex that controls transcription of DNA, cytokine production and cell survival. NF-kB regulates multiple aspects of innate and adaptive immune functions. It plays a central role in the inducible expression of inflammatory genes during the immune response, and the proper regulation of these genes is a critical factor in the maintenance of immune homeostasis [40]. Inflammation is a protective immune response of the host to infection and tissue damages [41] and normally it is beneficial to the host and can be resolved in a timely manner however deregulated inflammatory response can induce long lasting tissue damages contributing to the development of chronic inflammatory diseases such as UC.

Table 3. Hemoglobin, plasma iron, total iron binding capacity, transferrin saturation%, soluble transferrin receptors and erythroferrone of the different experimental groups

Groups	Hemoglobin (g/dl)	Iron µg/dL	TIBC µg/dL	Transferrin saturation%	Soluble transferrin receptor (ng/ml)	Erythroferrone (pg/ml)
NC	15.70^{c} ±0.31	90.73^{c} ± 3.19	200.51^{c} ±2.84	45.25^{b} ± 2.24	0.41^{a} ±0.01	1.38^{b} ±0.28
UCC	10.25^{a} ±0.57	50.77^{a} ± 1.58	258.21^{b} ± 5.43	19.66^{a} ± 0.59	0.73^{b} ±0.03	2.29^{a} ±0.52
Date seed	13.50^{b} ±0.51	79.5^{b} ± 1.17	148.96^{a} ± 5.49	53.37^{c} ± 1.97	0.56^{c} ±0.02	1.23^{b} ±0.12
Grape seed	12.47^{b} ±0.59	77.79^{b} ± 2.15	155.09^{a} ± 6.19	50.16^{c} ± 3.08	0.53^{c} ±0.01	1.21^{b} ±0.28

NC: Normal control, UCC; Ulcerative colitis control, Date seed: Group treated with date seed extracts, Grape seed: Group treated with grape seed extracts.
In each column same letters mean non-significant difference while different letters mean significant difference at $p< 0.05$. Data are expressed as mean values ± standard error.

Table 4. Colon MDA, NO and GSH and plasma ALP of the different experimental groups.

Groups	ALP (IU/L)	MDA (nmol/g. tissue)	NO (µmol/g. tissue)	GSH mg/g.tissue
NC	125.73^{a}±4.16	19.38^{a}±1.68	1.96^{a}±0.19	7.7^{bc} ± 0.68
UCC	169.79^{d}±2.44	32.36^{c}±1.74	5.04^{c}±0.47	2.68^{a} ± 0.71
Date seed	137.80^{bc}±3.42	26.48^{b}±1.16	3.20^{b}±0.27	9.24^{bc} ± 0.87
Grape seed	140.65^{c}±2.41	27.56^{b}±1.68	3.65^{b}±0.37	7.54^{bc}± 0.42

NC: Normal control, UCC; Ulcerative colitis control, Date seed: Group treated with date seed extracts, Grape seed: Group treated with grape seed extracts. ALP: Alkaline phosphatase, MDA: Malondialdehyde, NO: Nitric oxide, GSH: Reduced glutathione. In each column same letters mean non-significant difference while different letters mean significant difference at $p< 0.05$. Data are expressed as mean values ± standard error.

Table 5. Plasma INF-gamma and colon IL-6 of the different experimental groups

Groups	INF-gamma (pg/ml)	IL-6(pg/100mg colon tissue)
NC	340.2^{a}±5.46	77.72^{a}±2.58
UCC	337.59^{a}±5.59	153.72^{b}±5.54
Date seed	343.41^{a}±6.53	81.57^{a}±3.43
Grape seed	333.35^{a}±4.59	83.94^{a}±3.47

NC: Normal control, UCC; Ulcerative colitis control, Date seed: Group treated with date seed extracts, Grape seed: Group treated with grape seed extracts. INF-gamma: Interferon-gamma, IL-6: Interleukin-6
In each column same letters mean non-significant difference while different letters mean significant difference at $p< 0.05$. Data are expressed as mean values ± standard error.

Table 6. The relative expression of iNOS,COX2, IL-1β and NF-*k*Bgenes in colon tissue of the different experimental groups*

Groups	Relative expression			
	NF-*k*B	iNOS	COX2	IL-1β
NC	0.046±0.021[a]	0.021±0.006 [a]	0.107±0.012 [a]	0.344±0.157 [a]
UCC	1.000±0.383[b]	1.000±0.158[b]	1.000±0.200[b]	1.000±0.138[b]
Date seed	0.048±0.037[a]	0.199±0.022[ac]	0.593±0.028[c]	0.229±0.146 [a]
Grape seed	0.086±0.043[a]	0.243±0.053[c]	0.410±0.078[c]	0.403±0.061 [a]

NC: Normal control, UCC; Ulcerative colitis control, Date seed: Group treated with date seed extracts, Grape seed: Group treated with grape seed extracts, NF-*k*B: Nuclear factor kappa light chain enhancer of activated B cells, iNOS: Inducible nitric oxide synthase, COX2: Cyclooxygenase-2, IL-1β: Interleukin-1β. In each column same letters mean non-significant difference while different letters mean significant difference at $p< 0.05$.
Data are expressed as mean values ± standard error.
The mRNA expressions of iNOS, COX2, IL-1β and NF-kB are normalized with housekeeping gene (GAPDH), values of the groups receiving date and grape seed extracts have significant fold changes from ulcerative-colitis control.

COX-2 is involved in the conversion of arachidonic acid to prostaglandin H2 which is expressed in inflammation [42], therefore its elevation in the UCC group in the present study confirms the inflammatory state in UC. IL-1β is a member of the interleukin 1 family; the later include 11 cytokines that play a central role in the regulation of immune and inflammatory response. IL-1β is a cytokine protein which is a mediator of inflammatory response and is involved in cell proliferation, and apoptosis; so its increase in the colon of the UCC group pointed to inflammation and cell death of colon tissue. The induction of COX-2 by this cytokine is found to contribute to inflammatory pain hypersensitivity. IL-1β is suggested to be involved in modulation of autoimmune response [43] that has been postulated as one of the causative factors of UC. Also, IL-6 stimulates the inflammatory and autoimmune process in different diseases [44]. iNOS, COX2, IL-1β are regulated through NF-kB [22,45,46]. Studies have shown elevation in inflammatory cytokines in ulcerative colitis therefore iNOS, COX2, and IL-1β play an important role in the pathogenesis of inflammatory bowel diseases as pro-inflammatory mediators [47].

It could be noticed in Table, 6 that date and grape seed extracts significantly down-regulated the gene expression of COX-2 by 2 fold changes as compared to the UCC group and down-regulated the expression of IL-1β by 4.0 and 3.0, fold-changes, receptively. The mRNA expression of iNOS was significantly down-regulated on treatment with date seed extract (5 fold changes) and grape seed extract (4 fold changes). NF-kB gene expression was significantly down-regulated on receiving date seed extract by 2 fold changes while grape seed extract treatment significantly down-regulated the mRNA expression of NF-kB by 12 fold changes. The improvement of such inflammatory mediators in addition to IL-6 by the date and grape seed extracts indicated their therapeutic efficiency due to immune-regulating, anti-inflammatory and antioxidant effects. This immune-regulatory effect of both extracts may also reflect the improved iron status in such test groups.

Anemia was reported to present in UC, 57% of anemic UC patients are classified as having IDA [48]. Anemia was reported to be one of the most frequent comorbid condition associated with death in the inflammatory bowel diseases (IBD) and simply reflect the severity of the disease [49].The occurrence of anemia in UC might be related to both deregulation of immune system that results in elevated cytokine and the loss of blood from the inflamed colon. Anorexia may participate in the incidence of anemia in UC. Also, reduced iron and nutrients absorption is present in severe ulcerative colitis due to increased bowel movements, therefore sufficient absorption time is not present [30]. Patients with IBD (UC and crohn′s diseases) showed elevated hepcidin level although they have elevated erythroferrone. In IBD as in chronic inflammatory disease, inflammation increases hepcidin expression and synthesis because it is an acute phase protein [50]. This increased synthesis is partially mediated by IL-6, IL-1 and other cytokines. The increased hepcidin in IBD cause the retention of iron in macrophages and enterocytes leading to hypoferremia and iron restricted erythropoiesis with positive correlation between hepcidin and IL-6. This supports the hypothesis that IL-6 driven hepcidin production mediates anemia in IBD [51]. So, anti-inflammatory agents may play an important role in alleviating anemia. However in contrast other study showed reduced hepcidin in IBD [52] which may negate the aforementioned proposal. On the other hand, iron deficiency was reported to be associated with disorder in cell mediated and innate immunity [7]. It is now becoming clear that both the immune response and iron mutually and differentially interact with one another. That is, immunity plays an important role in managing intracellular iron and in responding to changing levels of systemic iron. On the other hand, iron and iron regulatory proteins modulate the immune response in different contexts [6]. In supporting this point, transferrin in macrophages and T lymphocytes has been reported to be essential for early T-cell differentiation [53,54]. Transferrin receptor 1 has been demonstrated to be critical for DNA synthesis and cell division in T-lymphocytes [55]. Also, macrophages and T lymphocytes have been reported to express significant levels of the iron storing protein ferritin [56,57].

Disease index of the different experimental groups represented by macroscopic examination of colon, feces consistency, bloody feces and percentage reduction of body weight gain are demonstrated in Table 7. It could be noticed that the UCC group has the greatest significant scores of disease index compared with the NC group and the treated groups. The elevated disease index in the UCC group agreed with previous studies [24-26]. Macroscopic examination of colon and feces consistency scores were significantly reduced when the group given date seed extract was compared to that received grape seed extract while the reverse occur concerning % reduction of body weight scores. Macroscopic examination of colon and bloody feces scores showed insignificant changes when the group treated with date extract was compared with NC group. Bloody feces scores of the group treated by grape seed extract showed insignificant change compared to the NC group. The disease index of the present study reflects

the superiority of date seed extract on grape seed extracts except for body weight gain reduction.

The histopathological examination of the colon (Fig1) showed mucosal erosion and ulceration, proliferation in lymphatic tissue, diffused inflammatory cells, mucosal crypts and hyperplasia of secretory epithelium and goblet cells in the UCC group compared to the NC group. The test groups showed only mild improvement in the histopathological changes of colon tissue. This result in comparison to the significantly improved biochemical parameters might indicate that histopathological changes might be improved if the study time was elongated so as the improved cytokines, NO, GSH and MDA may have an impact in tissue changes.

Table 8 showed the different fatty acids that present in date and grape seed petroleum ether extracts. The major fatty acid in date was oleic. Linoleic was the predominant fatty acid in grape seed. Grape seed oil only contained traces of linolenic acid. Total unsaturated fatty acids were higher than total saturated fatty acids in both grape and date seed petroleum ether extracts. Total unsaturated fatty acids in grape seed oil were higher than that in date seed oil. Phenolic profile of date and grape seed ethanol extract are present in Table 9. Variable phenolic compounds were present in both extracts. The major compounds in date seeds were chlorogenic acid, *p*-hydroxybenzoic acid, catechin and caffeic acid. In grape seed ethanol extract, catechin showed the highest level followed by protocatechuic acid, vanillic, syringic acid, *p*-hydroxybenzoic acid and gallic acid.

Grape seed was reported to contain both lipophilic and hydrophilic bioactive constituents including unsaturated fatty acids, carotenoids, phytosterols and toccopherols as lipophilic content as well as phenolic compounds as hydrophilic constituents such as resveratrol, quercetin in addition to procyanidins, tannins and stilbenes [58,59]. The main phenolic compounds in grape seed were demonstrated to be catechins, epicatechins, trans-resveratrol and procyanidin B1; catechins were the major phenolic compounds [60, 61] which agreed with the present study. Concerning the fatty acids in grape seed; linoleic was reported to be the most abundant where it constitute 66-75.3% of total fatty acids [62] which matched the results in the current study (65.52%). Linoleic acid is a polyunsaturated fatty acid that promotes human health [62, 63]. Oleic acid, a monounsaturated fatty acid is largely present in grape seed oil as cis form in a percentage of 19.57% of total fatty acids as could be seen from the present study which is more or less similar to that reported previously [64] that showed to be 14.3 %. Saturated fatty acids were present in low amount in grape seed as could be noticed from the present research and previous study [64]. Linolenic acid was only present in traces (0.35%) in the present study and in a previous one (0.15%) [64].

Date palm seeds were reported to contain cinnamic acid (and its derivatives), coumarins and phenolic compounds. Phenolic compounds present in the seeds are protocatechuic (7.9 mg/100g), caffeoyloshikimic (28.3 mg/100g), apigenin derivatives (0.5 mg/100g), quercetin derivatives (3.4 mg/100g), proanthocyanidines dimer (55.8 mg/100g), proanthocyanidines trimer (61.3 mg/100g) and epicatechin (18.8 mg/100g). These compounds were demonstrated to possess antioxidant, anti-inflammatory and immune-regulatory activities [65-68].

Table 7. Disease index: Macroscopic examination of colon, feces consistency, bloody feces (Mean± SE) and percentage reduction of body weight gain of the different experimental groups

Groups	Macroscopic examination of colon (scores)	Feces consistency scores	Bloody feces scores	Percentage reduction of body weight gain
NC	0.00^{a}± 0.00	0.00^{a}± 0.00	0.00^{a} ± 0.00	0%
UCC	3.14^{c}± 0.34	3.00^{b}± 0.3	2.00^{b} ± 0.3	28%
Date seed	0.86^{ab} ± 0.34	1.14^{c} ± 0.26	0.00^{a} ± 0.00	10%
Grape seed	1.43^{b} ± 0.43	2.00^{d}± 0.31	0.29^{a} ± 0.29	7%

NC: Normal control, UCC; Ulcerative colitis control, Date seed: Group treated with date seed extracts, Grape seed: Group treated with grape seed extracts.

In each column same letters mean non-significant difference while different letters mean significant difference at $p< 0.05$. Data are expressed as mean values ± standard error.

Macroscopic evaluation of colon: 0=no damage, 1=mucosal erythema, 2=mild mucosal edema, slight bleeding or erosion, 3=moderate edema, bleeding, erosion, ulcers, 4= severe edema, bleeding, erosion, ulcers

Feces consistency by scores: 0=Normal, 1=Soft (wet), 2= Very soft (semiliquid), 3= Watery diarrhea

Degree of bloody feces: 0=-ve, 1=Red, 2=Dark red, 3=Black

Weight gain loss: 0= -ve, 1=1-9% loss in body weight gain, 2=10-19% loss in body weight gain, 3= > 20% loss in body weight gain

Table 8. Fatty acid profile of date and grape petroleum ether extract as % of total fatty acids.

Fatty acids	Date (Relative %)	Grape (Relative %)
Lauric C12:0	**19.22**	**-**
Myristic C14:0	**11.56**	**-**
Palmitic C16:0	**10.54**	**8.91**
Stearic C18:0	**3.23**	**4.84**
Oleic C18:1	**44.74**	**-**
Cis isomer of oleic C18:1	**-**	**19.57**
Linoleic C18:2	**9.94**	**65.52**
α-linolenic C18:3	**-**	**0.35**
Arachidic C20:0	**0.33**	**-**
Eicosenoic C20:1	**0.44**	**-**
Total saturated fatty acids	**44.88**	**13.75**
Total unsaturated fatty acids	**55.12**	**85.44**

Table 9. Phenolic profile content of date and grape seed (µg/g) by HPLC

Compound	Date (µg/g extract)	Grape (µg/g extract)
Gallic acid	0	113.53
Protocatechuic acid	54.091	686.99
p-hydroxybenzoic acid	174.791	202.52
Gentisic acid	ND	ND
Catechin	103.708	1461.88
Chlorogenic acid	1045.183	ND
Caffeic acid	101.227	ND
Syringic acid	ND	260.78
Vanillic acid	ND	633.10
Ferulic acid	4.145	ND
Sinapic acid	ND	1.441
p-coumaric acid	ND	ND
Rutin	21.670	ND
Naringin	ND	ND
Rosmarinic acid	ND	ND
Cinnamic acid	ND	ND
Quercetin	ND	ND
Kaempferol	3.997	ND
Chrysin	ND	ND

ND: Not detecte

The result of the present study is similar to such study in containing protocatechuic and caffeic while it differs concerning the other phenolic compounds detected in the present study. The difference in constituents of date palm seeds is related to the difference in varieties of date palm. Fatty acids of date seed were reported to be lauric (14.2%), myristic (11.7%), palmitic (11.8%), oleic (47%) and linoleic (8.6%) [69]. The different percentages of fatty acids in the aforementioned study are similar to that of the present study except for arachidic and ecosaenoic that present in traces in the present study while were absent in the previous study.

The presence of phenolic compounds in both date and grape seed ethanol extract might impart an antioxidant, anti-inflammatory and immuno-regulatory activity [65,70] thereby improving ulcerative colitis and iron status. Petroleum ether extracts of date and grape seed, possess antioxidant and anti-inflammatory activity which was ascribed to the presence of vitamin E, phytosterols [59, 71] and unsaturated fatty acids that may synergize the action of the active constituents of the ethanol extract. Reduction in inflammation and

oxidative stress on treatment of date and grape seed extracts might reduce bleeding from the colon and thereby improve iron status.

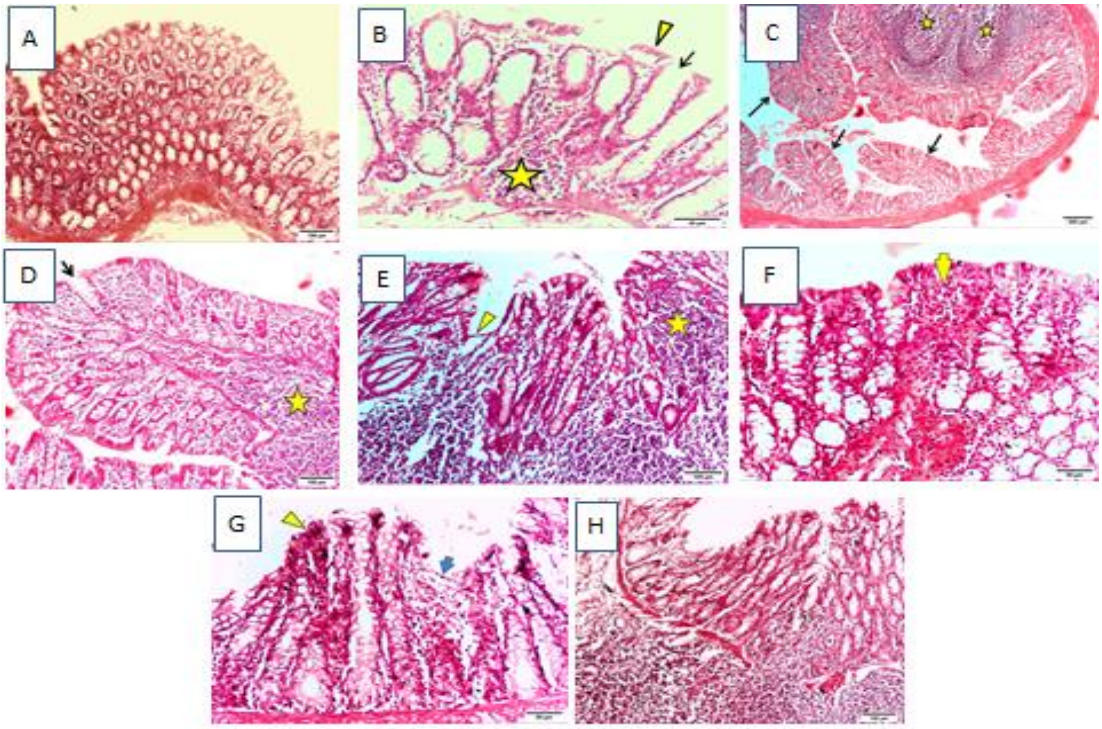

Figure 1: Representative images of H&E-stained colon sections of different experimental groups

A: Section of colon from NC group shows that the main architecture of colon tissue is within normal limit (X 100). **B-D**: Section of UCC colon, **B**: The section of colon illustrates activation of mucous secreting epithelium and glands (arrow), and focal erosion and ulceration of mucosa layer (arrowhead) associated with the proliferation of lymphatic tissues (star) (x 400), **C**: colon section demonstrates mild crypt distortion due to epithelial hyperplasia (arrows) associated with proliferation of lymphatic tissues (stars) (x 40), **D**: Section illustrates activation of mucous secreting epithelium and glands, with focal ulceration of mucosa layer (arrow) associated with the proliferation of lymphatic tissues (star) (x100). **E and F**: Sections of colon from rats treated by date seed extract, **E**: Section illustrates diffuse proliferation and invasion of the inflammatory cells to mucosa layer (star) and ulceration of mucosa layer (arrow head) (x 100), **F**: Colon section demonstrates focal necrosis of mucosa associated with inflammatory cell proliferation (x 200). **G and H:** Sections of colon from rats treated by grape seed extract, **G:** colon sections shows epithelial hyperplasia and nuclear hyperchromatic (arrow head) and ulceration (arrow) of mucosa (x200), **H:** Colon section illustrates diffuse proliferation and invasion of the inflammatory cells to mucosa layer associated with ulceration and erosion of mucosa cell layer.(x100). NC: Normal control, UCC: Ulcerative colitis control

Conclusion

The pathophysiology of iron deficiency and anemia in UC are important factors for the quality of life and deserve deep investigation in order to find an integrated care for UC patients. Further, therapies focused on acute phase inflammatory reactant might improve treatment option and outcome.

Within the extreme of the present study, iron deficiency anemia was shown to be interrelated to immune system through inflammatory cytokines like IL-1β and IL-6 and iNOS, COX2, and NF-*k*B genes expression but not IFNγ in UC rat model. Date and grape seed extracts showed variable significant improvements in UC through antioxidant, anti-inflammatory and immune-regulatory effects in addition to reducing IDA. Such effects might be ascribed to their phenolic contents and unsaturated fatty acids determined in the present study and other constituents like vitamin E, phytosterols and carotenoids reported in previous studies.

Conflict of interest

The authors declare that there is no conflict of interest

Acknowledgments

This research was completely financed and carried out in National Research Centre, Egypt (Grant No. 12060122).

References

1. Choi C.H., Jung S.A., Lee B.I., Lee K.M. and Kim J,S., Diagnostic guideline of ulcerative colitis.

The Korean Journal of Gastroenterology, **53**(3), 145-160 (2009)

2.Akiho H., Yokoyama A., Abe S., Nakazono Y., Murakami M., Otsuka Y., Fukawa K., Esaki M., Niina Y, and Ogino H., Promising biological therapies for ulcerative colitis: A review of the literature. *World Journal of Gastrointestinal Pathophysiology*, **6** (4), 219–27 (2015).

3.Bagatini M.D., Cardoso A.M., dos Santos A.A. and Carvalho F.B., Immune system and chronic diseases. *Journal of Immunology Research*, **2017**, Article ID 4284327, 3 pages, https://doi.org/10.1155/2017/4284327.

4.Rubinow K.B. and Rubinow D.R., In immune defense: redefining the role of the immune system in chronic disease. *Dialogues in Clinical Neuroscience*, **19**(1), 19-26 (2017)

5.Madu A.J. and Ughasoro M.D., Anaemia of chronic disease: An in-depth review. *Med. Princ. Pract*, **26**, 1–9 (2017).

6.Hamad M. and Bajbouj K., The re-Emerging role of iron in infection and immunity. *Integr Mol Med*, **3**(5), 807-810 (2016).

7.Ahluwalia N., Sun J., Krause D., Mastro A. and Handte G., Immune function is impaired in iron-deficient, homebound, older women. *Am J Clin Nutr*, **79** (3), 516-21 (2004).

8.Cherayil B. J., Iron and immunity: immunological consequences of iron deficiency and overload. *Arch Immunol Ther Exp (Warsz)*, **58**(6), 407–415 (2010).

9.Chen J.H., Andrews J.M., Kariyawasam V., Moran N., Gounder P., Collins G., Walsh A.J , Connor S. , Lee T.W.T. , Koh C.E. , Chang J. , Paramsothy S. , Tattersall S. , Lemberg D.A. , Radford-Smith G. , Lawrance I.C. , McLachlan A. , Moore G.T. , Corte C. , Katelaris P. and Leong R.W., Review article: acute severe ulcerative colitis - evidence-based consensus statements. *Alimentary Pharmacology & Therapeutics*, **44** (2), 127–44 (2016).

10.Feuerstein J.D., Isaacs K.L., Schneider Y., Siddique S.M., Falck-Ytter Y. and Singh S., AGA Clinical practice guidelines on the management of moderate to severe ulcerative colitis". *Gastroenterology*, **158** (5), 1450–1461 (2020).

11.Kim K.H., Tsao R., Yang R. and Cui SW., Phenolic acid profiles and antioxidant activities of wheat bran extracts and the effect of hydrolysis conditions. Food Chem, **95**, 466–73 (2006).

12.Qiu S., Li P., Zhao H. and Li X., Maresin 1 alleviates dextran sulfate sodium-induced ulcerative colitis by regulating NRF2 and TLR4/NF-kB signaling pathway. *International Immunopharmacol.*, **78**,106018. (2020). https://doi.org/10.1016/j.intimp.2019.106018

13.Bancroft J.D. and Gamble M., Theory and practice of histological techniques, Churchill Livingstone, Elsevier, China (2008).

14.Belfield A. and Goldberg D.M., Colorimetric determination of alkaline phosphatase activity. *Enzyme*, **12** (5), 561-568 (1971).

15.Volier M.A., Gschwind H. and Schlapfer P., Neue serumeisenbestimmung auf dem GSA II. *Lab Med*, **4**, 240-244 (1980).

16.Fairbanks V.F. and Klee G.G., Biochemical aspects of hematology In:Tietz MW.ed Fundamentals of clinical chemistry 3rd edition Philadelphia:WB saunders, p. 789-824 (1987).

17.Beutler E., Duron O. and Kelly M.B., Improved method for the determination of blood glutathione. *J Lab Clin Med*, **61**, 882-8 (1963).

18.Montgomery H.A.C. and Dymock J., The determination of nitrite in water. *Analyst*, **86**, 414-416 (1961).

19.Ohkawa H., Ohishi W. and Yagi K., Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem*, **95** (2), 351-8 (1979).

20.Khan H.A., Abdelhalim M.A.K., Alhomida A.S. and Al Ayed M.S., Transient increase in IL-1β, IL-6 and TNF-α gene expression in rat liver exposed to gold nanoparticles. *Genetics and Molecular Research*, **12** (4), 5851-5857 (2013).

21.Chun J.M., Kim H.S., Lee A.Y., Kim S.H. and Kim H.K., Anti-inflammatory and antiosteoarthritis effects of *Saposhnikovia divaricata* ethanol extract: *in vitro* and *in vivo* studies. *Evid-Based Complementary and Altern Med*, **2016**, Article ID 1984238, 8 pages (2016). https://doi.org/10.1155/2016/1984238

22.El-Ashmawy N.E., Khedr N.F., El-Bahrawy H.A. and El-Adaw S.A., Downregulation of iNOS and elevation of cAMP mediate the anti-inflammatory effect of glabridin in rats with ulcerative colitis. *Inflammopharmacol*, **26** (2), 551-559 (2018).

23.Livak K.J. and Schmittgen T.D., Analysis of relative gene expression data using real-time quantitative PCR and the 2–ΔΔCt method. *Methods*, **25**, 402–408 (2001).

24.Li X.P., Xu Y.J., Zhang C.L., Deng L. , Chang M. and Yu Z. , Dong Liu. Protective effect of calculus Bovis Sativus on dextran sulphate sodium-induced ulcerative colitis in mice. Evid Based Complement. *Alternat Med*., **2015**, 469506 (2015). doi: 10.1155/2015/469506.

25.Melgar S., Karlsson A. and Michaëlsson E., Acute colitis induced by dextran sulfate sodium progresses to chronicity in C57BL/6 but not in BALB/c mice: correlation between symptoms and inflammation. *Am J Physiol Gastrointest Liver Physiol*., **288**, G1328–G1338 (2005).

26.Süntar I., Cevik C.K., Çeribaşı A.O. and Gökbulut A. Healing effects of *Cornus mas* L. in experimentally induced ulcerative colitis in rats: From ethnobotany to pharmacology. *Journal of Ethnopharmacology*, 248, 112322 (2020). doi: 10.1016/j.jep.2019.112322. Epub 2019 Oct 20.

27.M.E. Street, De'Angelis G., Camacho-Hübner C., Giovannelli G, Ziveri M,A., Bacchini P.L., Bernasconi S., Sansebastiano G. and Savage M.O., Relationships between serum IGF-1, IGFBP-2, interleukin-1beta and interleukin-6 in inflammatory bowel disease. *Horm. Res. Paediatr.*, **61**, 159–164 (2004).

28.El-Mahdy N.A., Saleh D.A., Amerb M.S. and Abu-Risha SE., Role of allopurinol and febuxostat in the amelioration of dextran-induced colitis in rats. *European Journal of Pharmaceutical Sciences*, **141**, 105116 (2020). doi: 10.1016/j.ejps.2019.105116.

29.Martin J.C., Bériou G. and Josien R., Dextran sulfate sodium (DSS)-induced acute colitis in the rat. *Methods Mol Biol* ., **1371**, 197–203 (2016).

30.Rogler G. and Vavricka S., Anemia in inflammatory bowel disease: An under-estimated problem? *Front Med (lausanne)*, **1**, 58 (2014). doi: 10.3389/fmed.2014.00058. e Collection 2014

31.El Gendy F.M., El-Hawy M.A., Shehata A.M.F. and Osheba H.E., Erythroferrone and iron status parameters levels in pediatric patients with iron deficiency anemia. *Eur J Haematol*, **100** (4), 356-360 (2018[1]).

32.Coffey R. and Ganz T., Erythroferrone: An erythroid regulator of hepcidin and iron metabolism. *Hemasphere*, **2**(2): e35 (2018). doi: 10.1097/HS9.0000000000000035

33.Strole D.N. and Ganz T., Erythroferrone structure, function, and physiology: Iron homeostasis and beyond. *J. Cell Physiol.*, **236**, 4888–4901 (2021)

34.El-Gendy F.M., El-Hawy M.A., Rizk M.S., El-Hefnawy S.M. and Mahmoud M.Z., Value of soluble transferrin receptors and sTfR/log ferritin in the diagnosis of iron deficiency accompanied by acute infection. *Indian J Hematol Blood Transfus*, **34**(1), 104-109 (2018[2]).

35.Baillie F.J., Morrison A.E. and Fergus I., Soluble transferrin receptor: a discriminating assay for iron deficiency**.** *Clin Lab Haematol*, **25**(6), 353-7 (2003). doi: 10.1046/j.0141-9854.2003.00548.x.

36.Feelders R.A., Kuiper-Kramer E.P. and van Eijk H.G., Structure, function and clinical significance of transferrin receptors. *Clin Chem Lab Med*, **37** (1), 1-10 (1999).

37.Cook J.D., The measurement of serum transferrin receptor. *Am J Med Sci*, **318** (4), 269-276 (1999).

38.Al-Okbi S.Y., Mohamed R.S., Al-Siedy E.S.K. and Mohammed S.E., Functional foods for management of diarrhea and malnutrition in rats emphasizing on nucleotides role. *Recent Patents on Food, Nutrition & Agriculture*, **11** (3), 259-272(2020).

39.Kleinert H. and Forstermann U., Inducible nitric oxide synthase, In Editor(s): S.J. Enna, David B. Bylund, xPharm: The comprehensive pharmacology reference, Elsevier, p. 1-12 (2007).

40.Liu, T., Zhang, L., Joo, D. and Sun S-C,. NF-κB signaling in inflammation. *Sig Transduct Target Ther* , **2,** 17023 (2017). https://doi.org/10.1038/sigtrans.2017.23

41.Zhang H. and Sun S.C., NF-kappaB in inflammation and renal diseases. *Cell Biosci.*, **5**, 63 (2015). doi: 10.1186/s13578-015-0056-4. eCollection 2015.

42.O'Banion M.K., Cyclooxygenase-2: molecular biology, pharmacology, and neurobiology". *Crit Rev Neurobiol*, **13** (1), 45–82 (1999).

43.Sutton C.E., Lalor S.J., Sweeney C. M.; Brereton C.F.; Lavelle, Ed. C. and Mills K.H.G., Interleukin-1 and IL-23 induce innate IL-17 production from γδ T Cells, amplifying Th17 responses and autoimmunity. *Immunity*, **31** (2), 331–341 (2009). doi:10.1016/j.immuni.2009.08.001. ISSN 1074-7613. PMID 19682929

44.Swardfager W., Lanctôt K., Rothenburg L., Wong A., Cappell J. and Herrmann N., A meta-analysis of cytokines in Alzheimer's disease. *Biological Psychiatry*, **68** (10), 930-41 (2010).

45.Arab H.H., Al-Shorbagy M.Y., Abdallah D.M. and Nassar N.N., Telmisartan attenuates colon inflammation, oxidative perturbations and apoptosis in a rat model of experimental inflammatory bowel disease. *PLoS ONE*, **9**(5), e97193 (2014). doi:10.1371/journal.pone.0097193

46.Zaidi D. and Wine E., Regulation of Nuclear Factor Kappa-Light-Chain-Enhancer of Activated B Cells (NF-κβ) in Inflammatory Bowel Diseases. *Front.Pediatr* ., **6**, 317 (2018). doi: 10.3389/fped.2018.00317. eCollection 2018.

47.Ghasemi-Pirbalutia M., Motaghib E., Najafic A. and Hosseinic MJ., The effect of theophylline on acetic acid induced ulcerative colitis in rats. *Biomedicine & Pharmacotherapy*, **90**, 153–159 (2017).

48.Filmann N., Rey J., Schneeweiss S., Ardizzone S., Bager P., Bergamaschi G., Koutroubakis I., Lindgren S., de la Morena F., Moum B., Vavricka S.R., Schröder O., Herrmann E. and Blumenstein I., Prevalence of anemia in inflammatory bowel diseases in European countries: a systematic review and individual

patient data meta-analysis. *Inflamm Bowel Dis.*, 20(5), 936–45 (2014).
49.Cucino C. and Sonnenberg A., Cause of death in patients with inflammatory bowel disease. *Inflamm Bowel Dis.*, 7(3), 250–5 (2001).
50.Nemeth E., Valore E.V., Territo M., Schiller G., Lichtenstein A. and Ganz T., Hepcidin, a putative mediator of anemia of inflammation, is a type II acute-phase protein. *Blood* , **101**(7), 2461-3 (2003).
51.Basseri R.J., Nemeth E.,Vassilaki M.E., Basseri B., Enayati P., Shaye O., et al. Hepcidin is a key mediator of anemia of inflammation in Crohn's disease. *J Crohns Colitis*, 7(8), e286–91 (2013).
52.Mecklenburg I.,Reznik D., Fasler-Kan E., Drewe J., Beglinger C., Hruz P, et al. Serum hepcidin concentrations correlate with ferritin in patients with inflammatory bowel disease. *J Crohns Colitis*, **8**(11), 1392–7 (2014).
53.Nishiya K., Chiao J.W. and De Sousa M., Iron binding proteins in selected human peripheral blood cell sets: immunofluorescence. *Br J Haematol.*, **46**, 235-245 (1980).
54.Macedo M.F., de Sousa M., Ned R.M., Mascarenhas C., Andrews N.C. and Correia-Neves M., Transferrin is required for early T-cell differentiation. *Immunology*, **112**, 543-549 (2004).
55.Neckers L.M. and Cossman J., Transferrin receptor induction in mitogen-stimulated human T lymphocytes is required for DNA synthesis and cell division and is regulated by interleukin 2. *Proc Natl Acad Sci, USA*, **80**, 3494-3498 (1983).
56.Dörner M.H., Silverstone A., Nishiya K., de Sostoa A., Munn G., and de Sousa M., Ferritin synthesis by human T lymphocytes. *Science*, 209, 1019-1021 (1980).
57.Vezzoni P., Levi S., Gabri E., Pozzi M.R., Spinazze S. and Arosio P., Ferritins in malignant and non-malignant lymphoid cells. *Br J Haematol* ., 62, 105-110 (1986).
58. Duba K.S. and Fiori L., Supercritical CO_2 extraction of grape seed oil: effect of process parameters on the extraction kinetics. *J Supercrit Fluids*, **98,** 33–43 (2015).
59.Garavaglia J., Markoski M.M., Oliveira A., and Marcadenti A., Grape seed oil compounds: Biological and chemical actions for health. *Nutr Metab Insights*, **9**, 59–64 (2016).
60.Maier T., Schieder A., Kammerer D.R. and Reinhold C**.,** Residues of grape (Vitis vinifera L.) seed oil production as a valuable source of phenolic antioxidants. ***Food Chem*, 112**, 551–559 (2009).
61.Rombaut N., Savoire R., Thomasset B, Bélliard T., Castello J., Van Hecke E. and Lanoisellé J-L., Grape seed oil extraction: interest of supercritical fluid extraction and gas-assisted mechanical extraction for enhancing polyphenol co-extraction in oil. *Comptes Rendus Chimie*, **17** (3), 284–292 (2014).
62.Lutterodt H., Slavin M., Whent M., Turner E. and Yu L.L., Fatty acid composition, oxidative stability, antioxidant and antiproliferative properties of selected cold-pressed grape seed oils and flours. *Food Chem*, **128**(2), 391-9 (2011).
63.Shinagawa F.B., Santana F.C. and Mancini-Filho J., Effect of cold pressed grape seed oil on rats biochemical markers and inflammatory profile. *Rev. Nutr.*, **28**(1), 65–76 (2015).
64.Orsavova J., Misurcova L., Ambrozova J.V., Vicha R. and Mlcek J., Fatty Acids Composition of Vegetable Oils and Its Contribution to Dietary Energy Intake and Dependence of Cardiovascular Mortality on Dietary Intake of Fatty Acids. *Int J Mol Sci*, **16**(6):12871-90 (2015).
65.Vayalil P.K. Date fruits (*Phoenix dactylifera* Linn): an emerging medicinal food. *Crit. Rev. Food Sci. Nutr*., **52**(3), 249-71 (2012). doi: 10.1080/10408398.2010.499824.
66.Zavrˇsnik D., Muratovi´c S., Makuc D., Plavec J., Cetina M., Nagl A., De Clercq E., Balzarini J. and Mintas M., Benzylidene-bis- (4-hydroxycoumarin) and benzopyrano-coumarin derivatives: synthesis, 1H/13C-NMR conformational and X-ray crystal structure studies and in vitro antiviral activity evaluations," *Molecules*, **16** (7), 6023–6040 (2011).
67.El-Far A.H., Ahmed H.A. and Shaheen H.M., Dietary supplementation of *Phoenix dactylifera* seeds enhances performance, immune response, and antioxidant status in broilers. *Oxid Med Cell Longev*, 5454963 (2016). doi: 10.1155/2016/5454963 9pages
68.Benmeddour Z., Mehinagic E., Le Meurlay, D. and Louaileche H., Phenolic composition and antioxidant capacities of ten Algerian date (*Phoenix dactylifera* L.) cultivars: a comparative study. *J Func Foods*, **5** (1): 346–354 (2013).
69. Al-Shahib W., Marshall R.J. Fatty acid content of the seeds from 14 varieties of date palm *Phoenix dactylifera* L., *Int J Food Sci Technol*., **38** (6), 709–712 (2003).
70. Al-Okbi S.Y., Mabrok H.B., Al-Siedy K.S.K., Mohamed R.S. and Ramadan AA., Iron status, immune system, and expression of brain divalent metal transporter 1 and dopamine receptors D1 interrelationship in Parkinson′s disease and the role of grape seed and green coffee bean extracts and quercetin in mitigating the disease in rats. *J Herbmed Pharmacol.*, (in press).
71.Abd El-Wahab S.M., El Fiki N.M., El Sakhawy F.S. and Hassan A.B., Study of hormonal steroids

in *Phoenix dactylifera* L. seeds. *Bull Fac Pharm Cairo Univ*, **35,** 37 (1996).