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Protective and Therapeutic Roles of *Phoenix dactylifera* L. Zaghloul Cultivar Leaves Extract against Ethanol-induced Gastric Ulcer in Rats

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Abstract

Gastric ulcer is a common gastrointestinal disorder characterized by oxidative stress, inflammation, and mucosal injury. This study investigated the gastroprotective and therapeutic effects of *Phoenix dactylifera* (Zaghloul) methanolic leaves extract against ethanol-induced gastric ulcer in rats, with a focus on biochemical, molecular, and histopathological outcomes. Phytochemical analysis revealed that the extract is rich in phenolic (2450.127 mg GAE/g) and flavonoid compounds (2230.00 mg CE/g), known for their antioxidant and anti-inflammatory activities. Ethanol administration resulted in significant oxidative stress evidenced by decreased total antioxidant capacity (TAC) and glutathione (GSH) levels and elevated malondialdehyde (MDA). Treatment with the extract restored TAC and GSH and reduced MDA levels. TNF- α and ICAM-1 were significantly elevated in the ulcerated rats while significantly reduced in the extract treated rats; TNF- α /IL-10 ratio was increased in the ulcerative group and reduced by treatment. Moreover, the extract modulated the expression of genes associated with cellular stress and tissue repair including E2F1, RAD21, NIBPL and Vimentin suggesting attenuation of epithelial injury and involvement in mucosal regeneration. Histopathological examination confirmed the biochemical and molecular findings demonstrating improved mucosal architecture, enhanced mucin production, and reduced fibrosis. Collectively, these results reveal the impact of ulcer induction and treatment on E2F1, RAD21, NIBPL and Vimentin and point out the gastroprotective and therapeutic effects of *P. dactylifera* leaves extract through its modulation of the expression of these genes along with its antioxidant, anti-inflammatory and mucosal healing properties highlighting its potential as a natural therapeutic agent against gastric ulceration.

Keywords: *Phoenix dactylifera*, gastric ulcer, gastroprotective, antioxidant, anti-inflammatory, IL-10, TNF- α , phenolics, flavonoids, TNF- α /IL-10 ratio, E2F1, RAD21, NIBPL and Vimentin

Introduction

Gastric ulcer (GU) persists as prevailing gastrointestinal disorder worldwide that has significant negative effects on all aspects of life ¹. Moreover, untreated ulcers establish serious risk factors for severe health issues involving bleeding, perforation as well as gastric cancer (GC). However, gastric ulcer (GU) increases by millions every year globally indicating low quality management of this issue ². Therefore, GU research rapidly develops focusing on the identification of new GU molecular mediators as well as new approaches for GU treatment and prevention ³.

GU is a deep injury of the gastric mucosa that if not cured may reach deeper layers causing various adverse health effects and lowering the quality of the life of the GU patient ⁴. The mucosal layer of the gut is kept intact by many factors including the mucous secretion, epithelial barrier, blood flow, prostaglandins and bicarbonate ⁵. The elevation of the mucous destructive factors over the protective factors is a fundamental cause of GU that leads to the failure of the mucosal defensive barrier and the development of GU^{6,7}. This is because the mucosal defensive barrier failure augments proliferation of the inner epithelial cells and interrupts tight junctions causing rupture of the epithelial layer, infiltration of the gastric fluids to deep submucosal layers causing their erosion and leading to ulcer formation ⁸.

The imbalance between the pro-inflammatory and anti-inflammatory cytokines is a significant cause of GU. It activates neutrophils and impedes the antioxidant protective mechanisms ⁹. Also, the elevated production of reactive oxygen species (ROS) by the accumulated monocytes and neutrophils activate the lipid peroxidation and protein oxidation processes that cause cellular apoptosis and ulcer formation ¹⁰. ROS as well as the pro-inflammatory cytokines also induce apoptosis of the vascular endothelial cells of the gastric mucosa ¹¹. Furthermore, untermiated inflammation can lead to GC progression ¹².

Cyclooxygenase enzymes (COX) play fundamental role in the protection of the mucus layer. They enhance the production of prostaglandins (PGs) which stimulate mucous

and bicarbonate production, retain blood flow and enhance cells repair. Therefore, inhibition of COX enzymes is an important cause of GU that depletes the gastric mucosa protective factors¹³.

It is well known that the cell cycle is a fundamental process involved in homeostasis and tissue repair. Furthermore, failure or dysregulation of the cell cycle can lead to the progression of cancer. DNA damage is a principal leading cause of cancer especially if the cell cycle proceeded without DNA damage repair. Chromosomal instability is another cause of cell cycle dysregulation being a hallmark of the abnormally proliferating cancer cells¹⁴. Although untreated ulcer is a significant root of gastric cancer (GC)², many regulators of the cell cycle and DNA damage repair, that were implicated in cancer progression¹⁵, have not yet been studied in ulcer formation or treatment.

Vimentin (VIM) is an important regulator of the inflammatory responses and ROS production¹⁶. It is a member of the intermediate filaments (IFs) family that is found in mesenchymal derived cells, participates in the cytoskeleton formation and cellular homeostasis and determines the shape and mechanics of the cell¹⁷. VIM serves many cellular and physiological processes involving cell morphology, plasticity, migration, wound healing, tissue repair and immune system activation^{18,19}. However, the VIM function in intestinal disorders is poorly identified²⁰.

The Early 2 factor(E2F) proteins are fundamental transcription factors that modulate the cell cycle through various actions including the enhancement of DNA repair and replication²¹. This family has been known to have a significant role in the detection and repair of damaged DNA²². It involves eight members of which E2F1 is the most studied^{23,24}.

A cluster of conserved proteins known as cohesins serves to hold each two sister chromatids together until their separation during the anaphase (**Xu et al., 2015**)²⁵. These include the RAD21 protein which was known to be involved in DNA damage repair and inflammation modulation. RAD21 plays various additional biological processes including centrosome biogenesis, gene transcription, cell apoptosis and hematopoiesis.

Its deregulation has been found to be involved in the progression of multiple cancers^{26,27}.

Nipped-B- like protein (NIPBL) is a Cohesin loading factor found in the nuclei of all eukaryotic cells and functions mainly in loading cohesin onto chromosomes. It is also involved in the regulation of gene expression²⁸ and its mutation was identified in the Cornelia de Lange syndrome (CdLS), a developmental disorder characterized by many abnormalities including gastrointestinal abnormalities. Also, it was implicated in the development of various cancers including colorectal cancer²⁵.

Current medications for GU rely on anti-secretory regimens such as proton pump inhibitors, histamine-2 blockers and antacids. Although these drugs are effective in healing GU, they show low quality healing capacity, elevated recurrence rates and their long term use can cause adverse health effects^{29,30}. Therefore, several researches focus on medicinal plants as promising and safe alternatives with comprehensive healing capability and fewer adverse effects^{1,31}. Numerous plant derivatives hold anti-ulcer activities such as reducing gastric acid release, improving mucosal protection, replenishing blood flow and displaying antioxidant features³².

Phoenix dactylifera L. is a date palm tree belonging to the Arecaceae family and commonly cultivated in North Africa and the Middle East. Different parts of this tree have been reported to have antioxidant, antibacterial and antifungal activities³³. The leaves on the contrary were deduced to be less beneficial and thereby little research have focused on its biological activities³⁴. A recent study revealed that the methanolic leaf extract of Zaghloul cultivar of the Egyptian date palm displayed strong in vitro antioxidant activity that was even higher than that shown by vitamin C³⁵. Another study released by **Diab et al.**³⁶ revealed protective effects of the methanolic extract of *Phoenix dactylifera* leaves against doxorubicin-induced renal and hepatic toxicities in rats. To the best of our knowledge, no literature investigated the anti-ulcer activity of Zaghloul leaf extract.

Therefore, this study aimed to assess the protective and therapeutic effects of the methanolic extract of Zaghloul date palm leaves on ethanol induced ulcer in male Wistar albino rats. The study focused on measuring the oxidative stress markers (MDA, TAC & GSH activity), COXI & COXII enzymes, the inflammatory markers (ICAM-1,

TNF- α & IL-10) as well as the expression levels of Vimentin, E2F-1, RAD21 & NIPBL.

Materials and Methods

Phytochemical investigation

Chemicals and reagents

All chemicals and solvents used in the present study were of analytical grade and purchased from Merck & Co., Inc. (USA) and Sigma-Aldrich (USA).

Plant material and extraction

Fresh leaves of *Phoenix dactylifera* L. (Zaghloul date palm) were collected in December 2025 from a private farm located approximately 60 km along the Alexandria–Cairo desert road, Egypt. Botanical identification of the plant material was carried out by Prof. Dr. Abdelhalim Muhamed (Flora Department, National Agriculture Centre, Egypt). A voucher specimen (No. JSAP-2019) was deposited for future reference. The collected leaves were shade-dried, finely powdered using a mechanical grinder, and stored in tightly closed containers until use. The dried powdered leaves (500 g) were exhaustively extracted with 80% methanol at room temperature through repeated maceration until complete extraction was achieved. The combined extracts were concentrated under reduced pressure at 45 °C using a rotary evaporator to obtain the crude methanolic extract, which was then stored in a refrigerator until further phytochemical and biological investigations.

Determination of total phenolic and flavonoid contents

The total phenolic content (TPC) of the extract was determined using the Folin–Ciocalteu reagent according to the colorimetric method. The absorbance of the developed blue color was measured spectrophotometrically, and the total phenolic content was calculated using a standard calibration curve of Gallic acid. The results were expressed as micrograms of gallic acid equivalents per gram of extract ($\mu\text{g GAE/g extract}$). Also, the total flavonoid content (TFC) was determined using a colorimetric assay based on the formation of a flavonoid–reagent complex. The absorbance was measured spectrophotometrically, and the flavonoid content was calculated using a calibration curve prepared with Catechin as the reference standard. The results were expressed as micrograms of catechin equivalents per gram of extract ($\mu\text{g CE/g extract}$).

All measurements were performed according to previously reported procedures (El Sawi et al., 2024)³⁵

Biological investigation

Animals and experimental design

Sample size:

Following Aboul Naser et al.³⁷; Abdoulrahman,³⁸ Wetchakula et al.³⁹ and Shareef et al.⁴⁰, this study involved 36 Wistar albino male rats weighing 120-140 g).

Dosages and route of administration

Ulcer was induced by a single oral dose of absolute ethanol (0.5 ml/100 g weight) given to 24 hours fasting rats³⁷. Zaghloul extract was given orally at a dose of 400 mg/kg³⁶. Omeprazole standard drug was given orally at a dose of 40mg/kg³¹.

Experimental groups

Rats were accommodated in laboratory for a week with free access to rat chow and water. Then, they were divided into 6 groups 6 rats each: Group1: the control group that received 1 ml distilled water daily for seven days, Group 2: ulcerative group that received 1 ml distilled water daily for six days and a single dose of ethanol at the 7th day, Group 3: extract group that was given the extract only for 7 days without ulcer induction or treatment. Group 4: protective group that received the extract daily for seven days, and one hour after the last dose was induced for ulcer. Group5: extract treated group that treated with the extract for 7 days after ulcer induction. Group6: standard Omeprazole (OM) group that was treated with Omeprazole for 7 days after ulcer induction.

At the end of experiment, blood samples were collected from the orbital plexus of the eye in clean centrifuge tubes, left to clot & centrifuged at 3000rpm for 15 minutes for serum separation. The serum was then transferred to clean Eppendorf tubes and stored at -80 for further investigations.

Then, rats were sacrificed by decapitation and, longitudinal sections from each stomach were stored at -80° C for gene expression analysis. Other stomach slices were fixed and stored in 10% formaldehyde for histopathological examinations.

Biochemical Analysis

Determination of oxidative stress markers

Total antioxidant capacity (TAC) as well as the oxidative stress markers: Lipid peroxidation (Malondialdehyde; MDA) and glutathione (GSH) were determined according to **Gawron et al.**⁴¹; **Buege and Aust**⁴² and **Moron et al.**⁴³, respectively.

Determination of ICAM-1

Serum levels of the intercellular adhesion molecule-1 (ICAM-1) were determined using a commercial enzyme-linked immunosorbent assay (ELISA) kit (RayBiotech, USA) according to the manufacturer's instructions. Serum samples were diluted 1:50, and 50 µL of each sample was added to antibody-precoated wells and incubated for 2 h. A labeled antibody specific for soluble ICAM-1 (sICAM-1) was then added, followed by substrate solution. Absorbance was measured at 450 nm. ICAM-1 concentrations were calculated from a standard curve generated by linear regression using sICAM-1 standards and expressed as ng/mL.

Determination of COX-I and COX-II

Serum levels of cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) were measured using commercial ELISA kits according to the manufacturer's instructions. Serum samples and standards were added to antibody-coated plates and incubated at 37 °C, followed by washing and incubation with enzyme-conjugated antibodies. After addition of substrate and stop solutions, absorbance was read at 450 nm using a microplate reader. COX-1 and COX-2 concentrations were calculated from standard curves and expressed as ng/L.

Determination of IL-10 and TNF-α

Serum inflammatory cytokines were measured through the use of a commercial enzyme-linked immunosorbent assay (ELISA) (BT LAB Bioassay Technology Laboratory) according to the manufacturer's instructions. ELISA assays for IL-10 and TNF-α were performed using a microplate reader at 450 nm (Thermo Scientific, Waltham, MA). A duplicate set of each sample was also assayed. A standard curve was

constructed using various dilutions of IL-10 and TNF- α standard preparation. The levels of these cytokines were calculated according to standard curves.

Analysis of gene expression of E2F1, RAD21, NIBPL and Vimentin genes

Isolation of RNA:

Stomach tissues were collected from different treated male rat groups. Total RNA was extracted using the TRIzol® reagent method (Invitrogen, Germany). Briefly, tissues were homogenized in liquid nitrogen and TRIzol®, followed by chloroform phase separation and isopropanol precipitation. The RNA pellet was washed with 75% ethanol, air-dried, and dissolved in DEPC-treated water. Genomic DNA was removed by treatment with RNase-free DNase I⁴⁴. RNA purity was assessed by the A260/A280 ratio (1.8–2.1), and integrity was confirmed by agarose gel electrophoresis of 28S and 18S rRNA bands⁴⁵. RNA samples were used immediately for reverse transcription or stored at –80 °C.

Reverse transcription (RT) reaction

First-strand cDNA was synthesized from stomach RNA using the RevertAid™ First Strand cDNA Synthesis Kit (MBI Fermentas, Germany). Total extracted RNA (5 µg) was reverse transcribed in a 20 µL reaction containing MgCl₂, 5× RT buffer, dNTPs, oligo(dT) primer, RNase inhibitor, and M-MuLV reverse transcriptase) (Hamza et al., 2015). Reverse transcription was performed at 25 °C for 10 min, 42 °C for 60 min, followed by enzyme inactivation at 99 °C for 5 min. The resulting cDNA was immediately used or stored on ice for subsequent real-time PCR analysis.

Real Time-Polymerase Chain Reaction (RT-PCR)

Quantitative real-time PCR was performed using a StepOne™ Real-Time PCR System (Applied Biosystems, Thermo Fisher Scientific, USA). Reactions were carried out in 25 µL volumes containing SYBR® Premix Ex Taq™, gene-specific forward and reverse primers, nuclease-free water, and cDNA template⁴⁶. Thermal cycling conditions consisted of an initial denaturation at 95 °C for 3 min, followed by 40 cycles of denaturation (95 °C for 15 s), annealing (60 °C for 30 s), and extension (72 °C for 30 s). Melt curve analysis was performed from 60 to 95 °C to confirm primer specificity. Relative gene expression was calculated using the $2^{-\Delta\Delta CT}$ method^{47,48}.

Primer sequences are listed in Table 1.

Table (1): Primers sequences used for qRT-PCR

Gene	Primer sequence	NCBI Reference
E2F1	F: GGAGGCTGGATCTGGAAACT	NM_001100778.1
	R: CAAGAAGCGTTTGGTGGTCA	
RAD21	F: CCCCAGAAGAACCTCCCAAT	NM_001025701.1
	R: CTCGCTGAAGACCATGAAGC	
NIBPL	F:ATCCATCCGGTTCAGTGTGT	NM_001427697.1
	R: CGGGATCTTTTAGGCACGTG	
Vimentin	F: TGACATTGAGATCGCCACCT	NM_031140.1
	R: TCATCGTGGTGCTGAGAAGT	
GAPDH	F: GAGACAGCCGCATCTTCTTG	NM_017008.4
	R: TGA CTGTGCCGTTGA ACTTG	

E2F-1: E2F transcription factor1; RAD21: Double-strand-break repair protein; NIBPL: Nipped-B-like protein; GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

Histopathological investigation

Stomach paraffin sections of 5 µm thick was prepared from the fixed stomach slices, stained with haematoxylin& eosin as well as PAS stain and examined under light microscope for detection of any pathological changes and determining the lesion score (Aboul Naser et al., 2020)³⁷.

Statistical Analysis

SPSS computer program was used for data analysis using one-way ANOVA test. Data was expressed as the mean ± SD or mean ± SEM accompanied by post hoc test at least significant difference (LSD) between groups at $p < 0.05$.

Results

Phytochemical investigation

Extraction yield

The dried powdered leaves (400 g) were extracted to yield 26 g of crude extract.

Total phenolic and flavonoid content

Quantitative phytochemical analysis of the Zaghloul cultivar revealed that the total leaf extract contained a high level of phenolic compounds, reaching 2450.127 µg gallic acid equivalents (GAE)/g extract. Similarly, the total flavonoid content of the extract was determined to be 2230.00 µg catechin equivalents (CE)/g extract.

Biological investigation

Oxidative Stress Markers

Table 2 shows that the extract group showed no significant differences with the control group regarding TAC (1.7 ± 0.24 , $p = 0.134$), GSH (23.03 ± 2.00 , $p = 0.117$) and MDA (7.26 ± 1.67 , $p = 0.304$). The ulcerative group exhibited significant reduction in the mean levels of TAC (0.62 ± 0.14 , $p < 0.003$) and GSH (11.43 ± 2.21 , $p < 0.01$) compared to the control group which showed average levels (1.38 ± 0.22) and (18.9 ± 1.29) of TAC and GSH, respectively. MDA produced by the ulcerative group (14.48 ± 1.74) displayed highly significant increase compared to that produced by the control group (8.67 ± 1.68) ($p < 0.002$). The average levels of TAC in the protective (1.2 ± 0.23 , $p < 0.05$), extract treated (1.32 ± 0.32 , $p < 0.005$) and OM treated (1.27 ± 0.25 , $p < 0.007$) groups were highly significantly higher than that in the ulcerative group (0.62 ± 0.14). The mean levels of GSH in the protective (26.67 ± 3.25), extract treated (21.89 ± 4.1) and OM treated (24.84 ± 3.87) groups were highly significantly higher than that in the ulcerative group (11.43 ± 2.2) ($p < 0.001$). MDA average levels were highly significant lower in the protective (9.4 ± 1.65) ($p < 0.003$), extract treated (9.25 ± 1.05) ($p < 0.003$) and OM treated (9.25 ± 1.05) ($p < 0.002$) groups than that in the ulcerative group (14.48 ± 1.74). No significant difference was found between the OM group and the protective or the extract treated groups

Table (2) the average levels of the oxidative stress markers (TAC, GSH & MDA) in the different groups

Group Biomarker	Control group	Ulcerative group	Extract group	protective group	Extract treated group	OM treated group
TAC (mM/L)	1.38±0.22 ^a	0.62±0.14 ^b	1.7±0.24 ^a	1.2±0.23 ^a	1.32±0.32 ^a	1.27±0.25 ^a
GSH (mg/dl)	18.93±1.29 ^a	11.43±2.2 ^b	23.03±2.00 ^a	26.67±3.25 ^a	21.89±4.1 ^a	24.84±3.87 ^a
MDA (nmol/ml)	8.67±1.68 ^a	14.48±1.74 ^b	7.26±1.67 ^a	9.4±1.65 ^a	9.25±1.05 ^a	8.29±1.73 ^a

ANOVA test with post hoc LSD. Significantly different groups have different characters (a or b).

ICAM-1

As shown in figure 1, the average level of ICAM-1 produced by the extract group (3.63±0.24) showed no statistical difference with that produced by the control group (3.87±0.27) ($p=0.557$). The mean concentration of ICAM-1 was increased significantly by 263.8% in the ulcerative group (10.21±0.38) compared to that of the control group (3.87±0.27) ($P<0.001$). On the other hand, the protective and extract treated groups showed highly significant lower concentrations of ICAM-1 (6.27±0.30, 6.86±0.28, respectively) than that produced by the ulcerative group (10.21±0.38) ($P<0.001$). The OM group showed highly significant decrease in concentrations of ICAM-1 (5.32±0.15) versus that in the ulcerative group (10.21±0.38) ($P<0.001$).

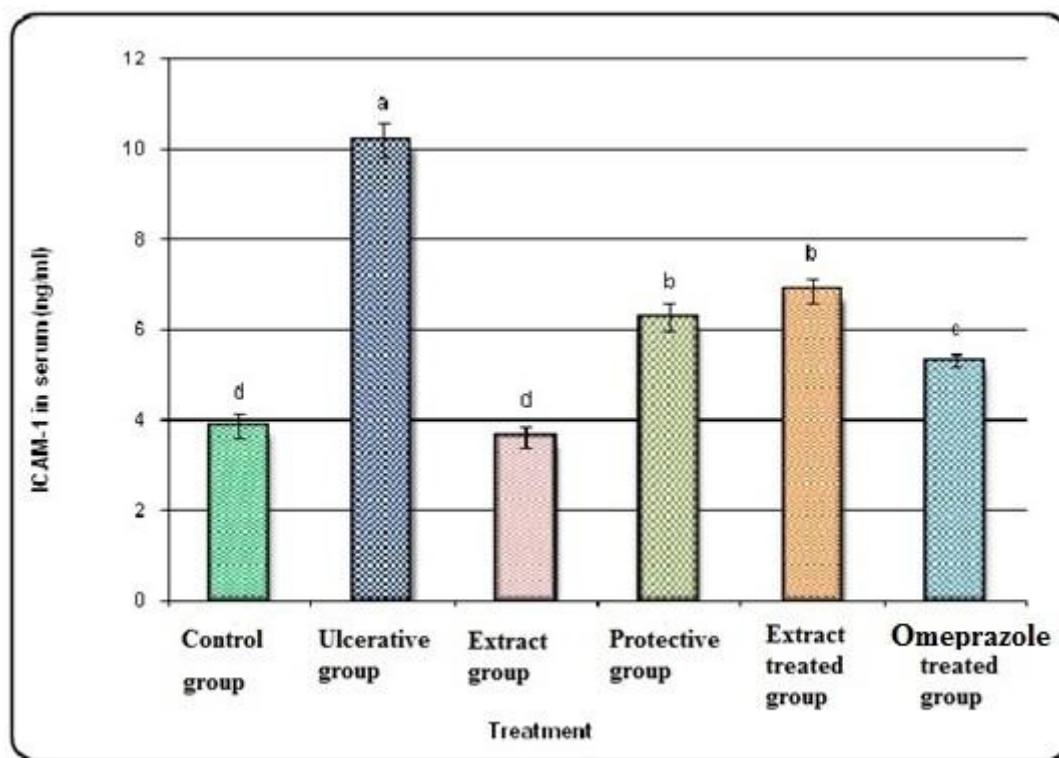
COX-1 & COX-2

The mean concentrations of COX-1 and COX-2 in the extract group (9.38±0.25 with $p=0.954$ and 12.45±0.38 with $p=0.914$, respectively) were not statistically different from those of the control rats (9.36±0.21 and 12.39±0.26, respectively). The average concentrations of COX-1 and COX-2 exhibited highly significant reduction by 40.7% and 42.5% in ulcerative rats (3.81±0.24 and 5.26±0.32, respectively) compared to those in control rats (9.36±0.21 and 12.39±0.26, respectively) ($p<0.001$). In contrast, the mean concentrations of COX-1 and COX-2 in the protective group (8.20±0.32 and 9.68±0.36, respectively) and in the extract treated group (7.08±0.35 and 7.51±0.37, respectively) showed highly significant increase compared with those in ulcerative rats (3.81±0.24 and 5.26±0.32, respectively) ($p<0.001$). Furthermore, OM treated rats exhibited significant raise in the average concentrations of COX-1 and COX-2

(8.31 ± 0.31 and 10.09 ± 0.39 , respectively) compared with those in ulcerative rats (3.81 ± 0.24 and 5.26 ± 0.32 , respectively) ($p < 0.001$), data are shown in figures 2&3.

IL-10 & TNF- α

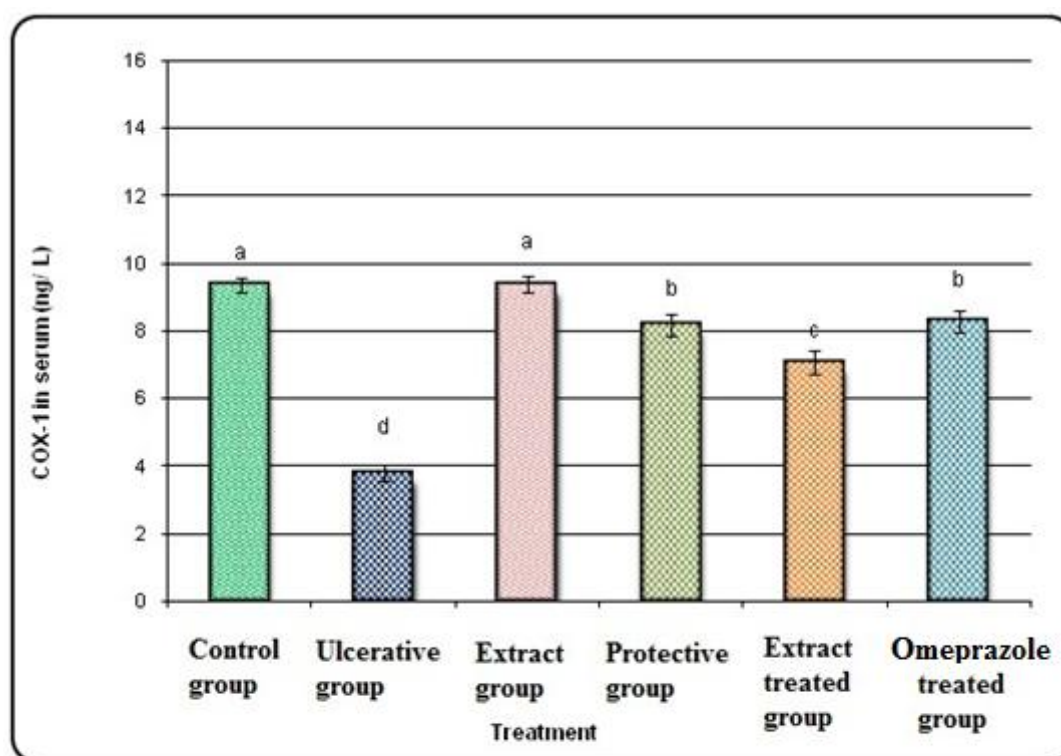
The average levels of IL-10 and TNF- α in the control rats (15.26 ± 0.57 and 55.43 ± 4.41 , respectively) were fairly like to those in rats treated with plant extract only (15.01 ± 0.49 and 53.69 ± 3.81 , respectively) ($p = 0.763$, $p = 0.799$, respectively). Contrariwise, the levels of IL-10 and TNF- α were increased significantly by 177.7% and 393.4% in the ulcerative rats (27.12 ± 0.81 and 218.06 ± 5.56 , respectively) compared to those in control rats (15.26 ± 0.57 and 55.43 ± 4.41 , respectively) ($p < 0.001$). The mean levels of IL-10 and TNF- α in the protective group (18.42 ± 0.54 and 123.27 ± 5.08 , respectively) and in the extract treated group (21.70 ± 0.61 and 143.54 ± 5.28 , respectively) showed highly significant drop compared with those in ulcerative rats (27.12 ± 0.81 and 218.06 ± 5.56 , respectively) ($p < 0.001$). Also, The OM treated group revealed significant decrease in the levels of IL-10 and TNF- α (17.54 ± 0.43 and 119.75 ± 4.59) compared with those in ulcerative rats (27.12 ± 0.81 and 218.06 ± 5.56 , respectively) ($p < 0.001$), data are illustrated in figures 4&5.



Treatment	Mean	SEM	(% of control)	(% of disease)	Inhibition
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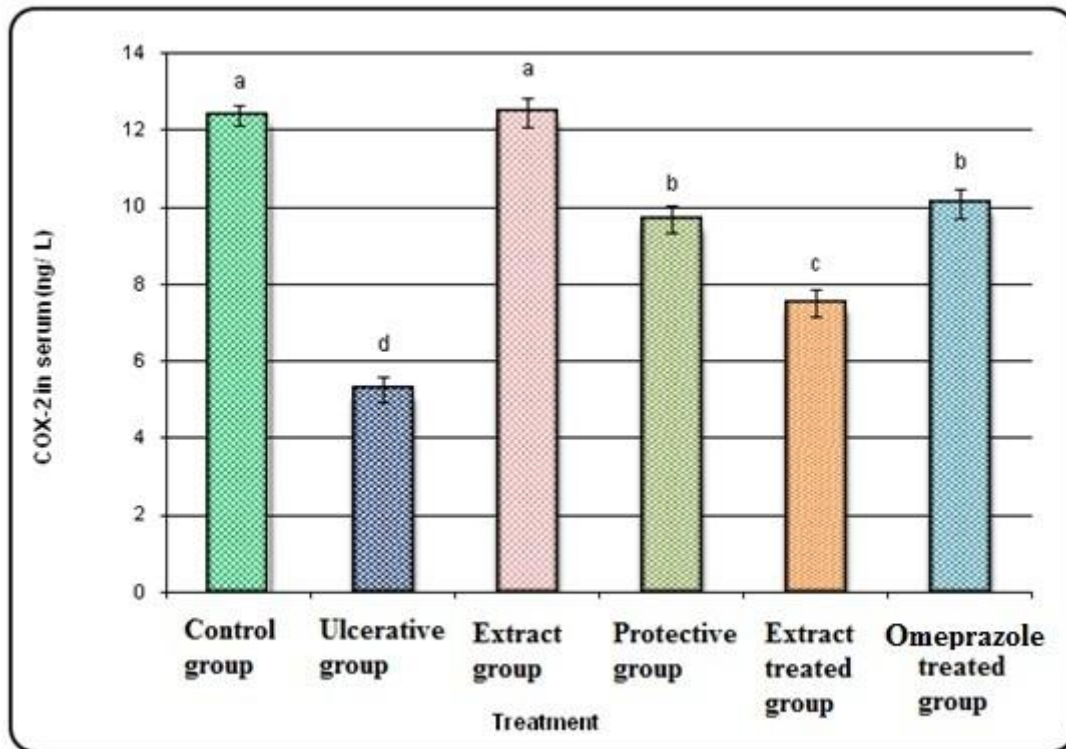
Control group	3.87	0.27	100.0	0.00	0.00
Ulcerative group	10.21	0.38	263.8	100.0	0.00
Extract group	3.63	0.24	93.8	35.6	64.4
Protective group	6.27	0.30	162.0	61.4	38.6
Extract treated group	6.86	0.28	177.3	67.2	32.8
OM treated (standard drug) group	5.32	0.15	137.5	52.1	47.9

Fig. 1: Mean concentrations of serum ICAM-1 in the different groups. Data are presented as mean $\pm$ SEM. Significantly different groups have different characters (a, b, c or d).



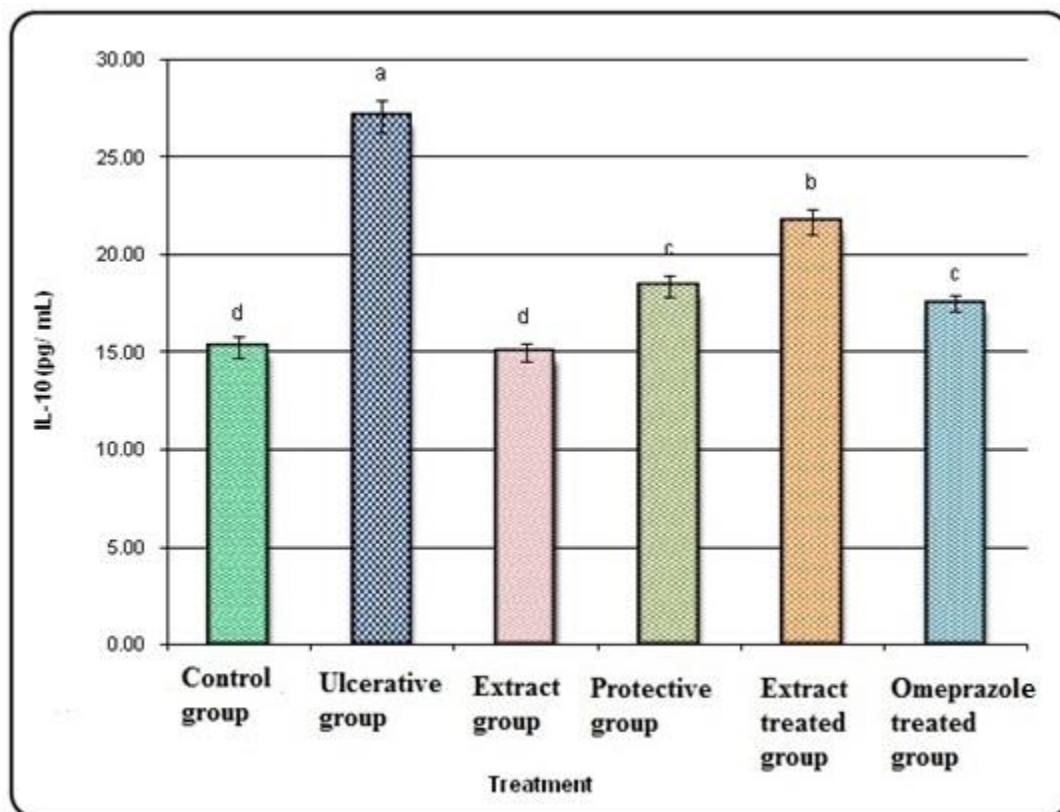
Treatment	Mean	SEM	(% of control)	(% of disease)	Inhibition
Control group	9.36	0.21	100.0	0.00	0.00
Ulcerative group	3.81	0.24	40.7	100.0	0.00
Extract group	9.38	0.25	100.2	246.2	146.2
Protective group	8.20	0.32	87.6	215.2	115.2
Extract treated group	7.08	0.35	75.6	185.8	85.8
OM treated (standard drug) group	8.31	0.31	88.8	218.1	118.1

Fig. 2: Mean concentrations of COX-1 in serum of different groups. Data are presented as mean $\pm$ SEM. Data are presented as mean $\pm$ SEM. Significantly different groups have different characters (a, b, c or d).



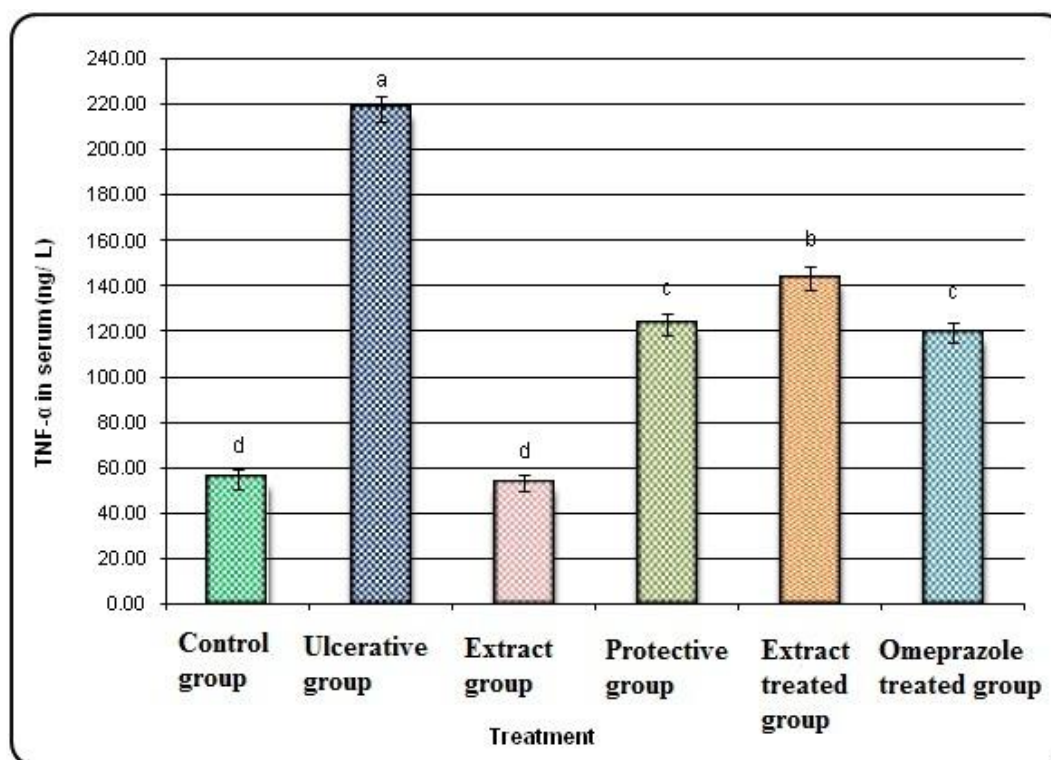
Treatment	Mean	SEM	(% of control)	(% of disease)	Inhibition
Control group	12.39	0.26	100.0	0.00	0.00
Ulcerative group	5.26	0.32	42.5	100.0	0.00
Extract group	12.45	0.38	100.5	236.7	136.7
Protective group	9.68	0.36	78.1	184.0	84.0
Extract treated group	7.51	0.37	60.6	142.8	42.8
OM treated (standard drug) group	10.09	0.39	81.4	191.8	91.8

Fig. 3: Mean concentrations of COX-2 in serum of the different groups. Data are presented as mean $\pm$ SEM. Data are presented as mean $\pm$ SEM. Significantly different groups have different characters (a, b, c or d).



Treatment	Mean	SEM	(% of control)	(% of disease)	Inhibition
Control group	15.26	0.57	100.0	0.00	0.00
Ulcerative group	27.12	0.81	177.7	100.0	0.00
Extract group	15.01	0.49	98.4	55.3	44.7
Protective group	18.42	0.54	120.7	67.9	32.1
Extract treated group	21.70	0.61	142.2	80.0	20.0
Omeprazole treated group	17.54	0.43	114.9	64.7	35.3

Fig. 4:Mean concentrations of IL-10 in serum of the different groups assessed by ELISA. Data are presented as mean $\pm$ SEM. Data are presented as mean $\pm$ SEM. Significantly different groups have different characters (a, b, c or d).



Treatment	Mean	SEM	(% of control)	(% of disease)	Inhibition
Control group	55.43	4.41	100.0	0.00	0.00
Ulcerative group	218.06	5.56	393.4	100.0	0.00
Extract group	53.69	3.81	96.9	24.6	75.4
Protective group	123.27	5.08	222.4	56.5	43.5
Extract treated group	143.54	5.28	259.0	65.8	34.2
OM treated (standard drug) group	119.75	4.59	216.0	54.9	45.1

Fig. 5: Mean concentrations of TNF- α in serum of different groups assessed by ELISA. Data are presented as mean $\pm$ SEM. S significantly different groups have different characters (a, b, c or d).

Average levels of E2F1, RAD21, NIBPL and Vimentin genes in Stomach tissues

E2F1

As shown in figure 6, the mean level of E2F1 expressed by the extract group (1.07 ± 0.06) was not statistically different from that expressed by the control group (1.00 ± 0.05) ($p = 0.447$). Highly significant increase by 154% (1.54 ± 0.09) was shown by the ulcer ulcerative group compared to the control rats (1.00 ± 0.05) ($p < 0.001$). The Extract treated group expressed an average amount of E2F1 (1.65 ± 0.09) that was not significantly different from that of the ulcerative group (1.54 ± 0.09) ($p = 0.261$). The mean levels of E2F1 expressed by the protective group (2.08 ± 0.08) and the OM group (2.28 ± 0.07) were highly significant elevated compared to that of the ulcerative group (1.54 ± 0.09) ($p < 0.001$).

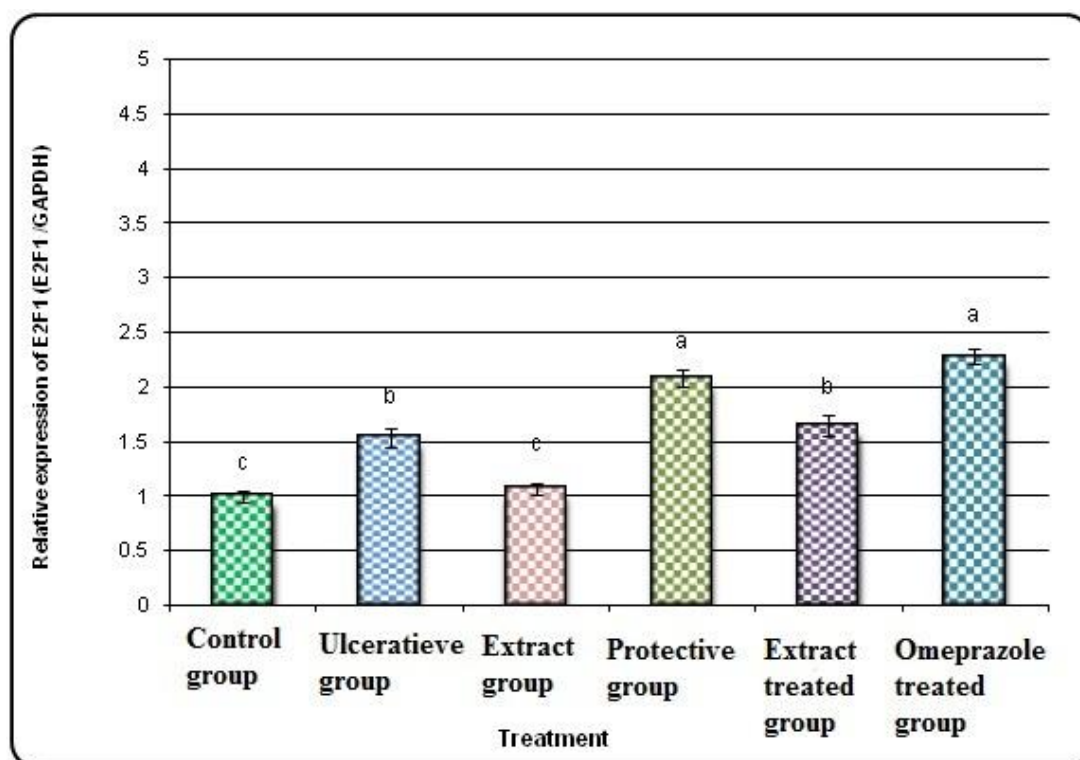
RAD21 and NIBPL

The mean expression levels of RAD21 and NIBPL genes in the stomach of the extract group (1.12 ± 0.058 and 1.11 ± 0.057 , respectively) were fairly similar to those of the control group (1.00 ± 0.057 and 1.00 ± 0.067 , respectively) ($p = 0.412$, $p = 0.417$, respectively). The ulcerative group exhibited highly significant elevation of the expression levels of RAD21 and NIBPL (1.74 ± 0.13 and 1.51 ± 0.15 , respectively) compared to the control rats (1.00 ± 0.057 and 1.00 ± 0.067 , respectively) ($p < 0.001$). The Extract treated group showed highly significant elevation in the expression levels of RAD21 (2.41 ± 0.11) and NIBPL (3.61 ± 0.13) compared to the untreated rats (1.74 ± 0.13 and 1.51 ± 0.15 , respectively) ($P < 0.001$). Additionally, the expression levels of RAD21 and NIBPL genes in the protective group (3.56 ± 0.09 and 3.93 ± 0.14 , respectively) and in the OM group (4.03 ± 0.10 and 4.61 ± 0.11 , respectively) revealed highly significant increase compared to those in untreated rats (1.74 ± 0.13 and 1.51 ± 0.15 , respectively) ($P < 0.001$), data are shown in figures 7&8.

Vimentin

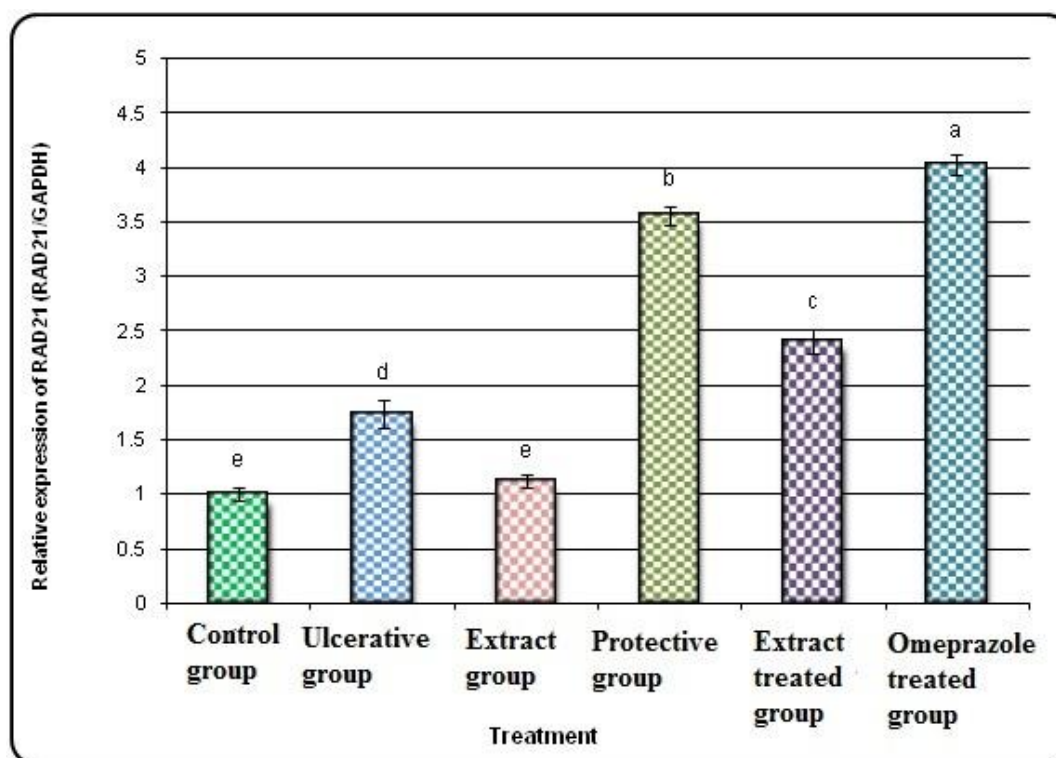
As shown in figure 9, the mean expression level of VIM gene in the extract group (1.15 ± 0.07) was close to that of the control group (1.00 ± 0.09) ($p = 0.388$). The ulcerative group showed highly significant increase (6.17 ± 0.16) compared to the control rats (1.00 ± 0.09) ($P < 0.001$). The extract treated group showed highly significant reduction in the mean expression level of VIM gene (3.51 ± 0.15) compared to the ulcerative group (6.17 ± 0.16) ($P < 0.001$). Also, the average expression levels of VIM

gene in the protective group (2.73 ± 0.11) and in the drug treated group (2.24 ± 0.10) revealed highly significant reduction compared with the ulcerative group (6.17 ± 0.16) ($P < 0.001$).



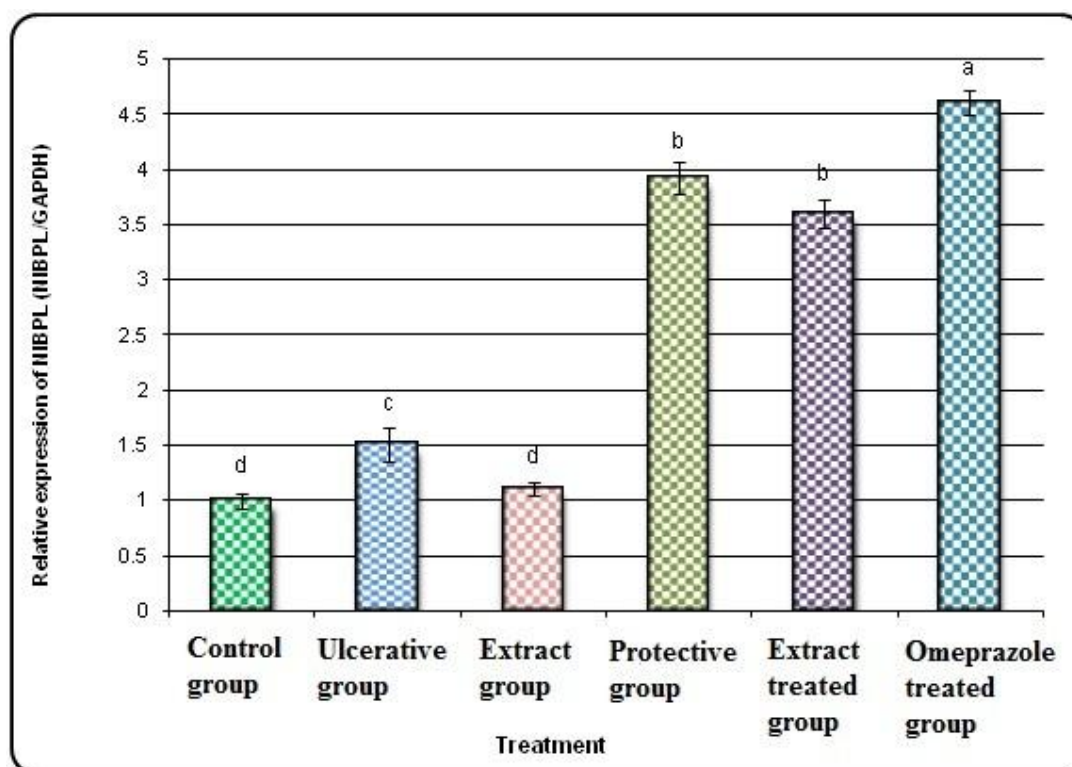
Treatment	Mean	SEM	(% of control)	(% of disease)	Inhibition
Control group	1.00	0.05	100	0.00	0.00
Ulcerative group	1.54	0.09	154	100.0	0.00
Extract group	1.07	0.06	107	69.5	30.5
Protective group	2.08	0.08	208	135.1	35.1
Extract treated group	1.65	0.09	165	107.1	7.1
OM treated (standard drug) group	2.28	0.07	228	148.1	48.1

Figure 6: The expression alterations of E2F1 gene in Stomach samples of the different groups. Data are presented as mean $\pm$ SEM. Data are presented as mean $\pm$ SEM. Significantly different groups have different characters (a, b, c or d).



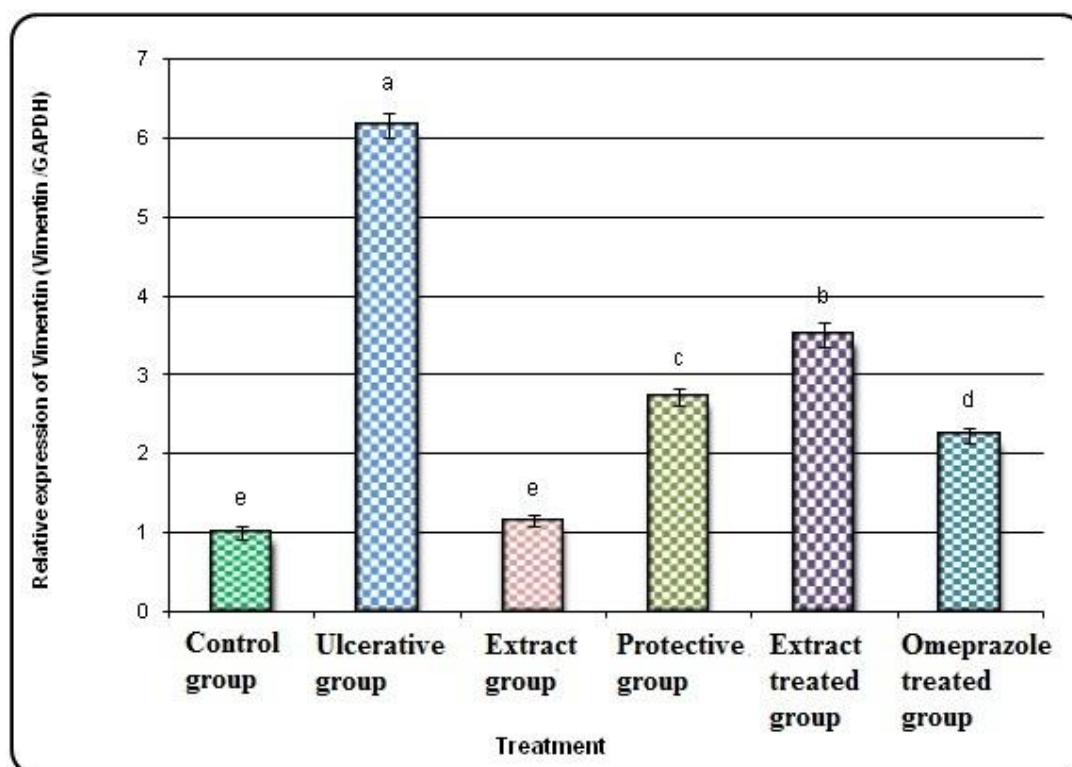
Treatment	Mean	SEM	(% of control)	(% of disease)	Inhibition
Control group	1.00	0.06	100	0.00	0.00
Ulcerative group	1.74	0.13	174	100.0	0.00
Extract group	1.12	0.06	112	64.4	35.6
Protective group	3.56	0.09	356	204.6	104.6
Extract treated group	2.41	0.11	241	138.5	38.5
OM treated (standard drug) group	4.03	0.10	403	231.6	131.6

Figure 7:The expression alterations of RAD21 gene in Stomach samples of the different groups. Data are presented as mean $\pm$ SEM. Data are presented as mean $\pm$ SEM. Significantly different groups have different characters (a, b, c or d).



Treatment	Mean	SEM	(% of control)	(% of disease)	Inhibition
Control group	1.00	0.07	100	0.00	0.00
Ulcerative group	1.51	0.15	151	100.0	0.00
Extract group	1.11	0.06	111	73.5	26.5
Protective group	3.93	0.14	393	260.3	160.3
Extract treated group	3.61	0.13	361	239.1	139.1
Omeprazole treated group	4.61	0.11	461	305.3	205.3

Figure 8:The expression alterations of NIBPLgene in Stomach samples treated of the different groups. Data are presented as mean $\pm$ SEM.Data are presented as mean $\pm$ SEM.Significantly different groups havedifferent characters (a, b, c or d).



Treatment	Mean	SEM	(% of control)	(% of disease)	Inhibition
Control group	1.00	0.07	100	0.00	0.00
Ulcerative group	4.38	0.15	438	100.0	0.00
Extract group	1.11	0.06	111	25.3	74.7
Protective group	2.32	0.14	232	53.0	47.0
Extract treated group	2.82	0.13	282	64.4	35.6
OM treated (standard drug) group	2.23	0.11	223	50.9	49.1

Figure 9: The expression alterations of Vimentingene in Stomach samples of the different groups. Data are presented as mean $\pm$ SEM. Data are presented as mean $\pm$ SEM. Significantly different groups have different characters (a, b, c or d).

Histopathological investigation

Histopathological examination of gastric sections revealed clear structural differences among the different experimental groups and control ones. The control group displayed completely normal gastric architecture which characterized by an intact columnar gastric epithelium, well-organized gastric glands and a thin, normal submucosal layer (Fig. 10A). On the other hand, the ulcerative(gastric ulcer) group showed severe pathological deteriorations including extensive mucosal destruction, epithelial and glandular degeneration, sloughing, ulceration and markedly thickened edematous submucosa infiltrated with lymphocytes and congested blood vessels (Fig. 10B).Comparing with the ulcerative group, the Omeprazole-treated group demonstrated substantial recovery of gastric tissue. The mucosa and glands appeared nearly normal and the general structure of submucosal layer appeared nearly normal although it is remained mildly thickened with lymphocytic infiltration which confirms the expected therapeutic effect of the standard drug (Fig. 10C).The extract group exhibited identical normal gastric structure with fully preserved mucosa and normal submucosal thickness which confirms the absence of any detectable tissue toxicity from the extract (Fig. 10D).The protective group displayed some degenerative changes appeared in the mucosa and glands, submucosal edema and vascular dilation but the injury was clearly milder than that of ulcerative group indicating partial or incomplete prevention of ulceration (Fig. 10E).The extract treated group showed nearly complete restoration of epithelial integrity and glandular architecture following ulcer induction. Submucosal edema and vascular dilation persisted to some extent while the mucosal covering appeared markedly improved compared to the ulcerative group and superior to the prophylactic group indicating strong therapeutic effect of the extract (Fig. 10F).

Examination of Periodic Acid–Schiff (PAS) stained gastric sections revealed differences in mucopolysaccharide content, reflecting mucosal integrity and mucin secreting cell activity. The control group demonstrated strong PAS positivity in the surface epithelium and moderate reactivity in gastric glands which is consistent with normal mucin production(Fig. 11A).The ulcerative group showed complete loss of PAS reactivity in both epithelium and glands due to extensive mucosal destruction confirming severe depletion of mucin-producing cells(Fig. 11B).The Omeprazole-treated group restored strong PAS positivity in the epithelium and moderate reactivity in glands which approach the normal distribution and reflect the tissue recovery(Fig.

11C).The extract group displayed strong epithelial and moderate glandular PAS staining confirming preserved mucin production in the absence of injury(Fig. 11D).The protective group exhibited reduced PAS reactivity which was moderate in epithelium and weak in glands, suggesting partial preservation of mucin despite ulcer induction. This aligns with the incomplete morphological protection seen in H&E study (Fig. 11E). The treatment group showed strong epithelial and moderate glandular PAS staining, indicating a notable restoration of mucin production. This profile closely resembled that of the Omeprazole group which supports the robust healing effect (Fig. 11F).

Examination of Masson's Trichrome stained gastric sections clarified changes in collagen deposition and submucosal remodeling. The control group exhibited a normal fibroelasticsubmucosa with typical vascularity and intact epithelial and glandular components(Fig. 12A).The ulcerative group displayed massive submucosal thickening with intense fibroelastic tissue accumulation and vascular congestion reflecting active fibrosis and inflammatory remodeling after ulceration (Fig. 12B).The Omeprazole-treated group demonstrated nearly normal submucosal organization with mild accumulation of fibroelastic tissue consistent with partial reversal fibrotic remodeling of ulcer (Fig. 12C).The extract group showed normal connective tissue structure similar to the control confirming the absence of fibrosis or pathological remodeling (Fig. 12D).Both the prophylactic and treatment groups showed normal gastric wall and markedly thickened submucosa with congested vasculature indicating that the extract promoted mucosal healing but did not fully reverse submucosal vascular congestion (Fig. 12E, F).

Histopathological scoring of gastric lesions demonstrated clear and statistically meaningful differences among the different experimental groups across all evaluated parameters (congestion, mucus depletion, inflammatory cell infiltration, epithelial injury, ulceration, and fibrosis) (Table 3). The scoring pattern strongly corroborates the qualitative histological findings. The control group showed a complete absence of pathological alterations, scoring 0 across all eight measured parameters, confirming the normal integrity of the gastric mucosa. In contrast, the ulcerative group exhibited the highest degree of tissue damage with markedly elevated scores across all categories. Congestion reached the maximum level (score 4), while mucus depletion, lymphocytic infiltration, epithelial degeneration, sloughing, ulceration and fibrosis all scored three

indicating 51–75% tissue damage. Neutrophil infiltration also showed a moderate score of two. This scoring profile confirms severe mucosal and submucosal injury and validates the ulcer induction model. The Omeprazole-treated group demonstrated substantial improvement showing minimal congestion and mucus depletion (score 1), absence of neutrophil infiltration (score 0), and only mild lymphocytic infiltration (score 1). Epithelial degeneration was absent, while sloughing scored only one. Ulceration and fibrosis both scored zero. This reflects a near-complete reversal of gastric damage and confirms the therapeutic effectiveness of the standard drug. The extract group exhibited a histopathological scoring pattern identical to the control with zero score across all parameters. This confirms that the extract does not induce any detectable gastric injury and is histologically safe when administered under normal conditions. The prophylactic group displayed a moderate protective effect, with mild congestion (score 1), moderate mucus depletion (score 2) and mild neutrophilic and lymphocytic infiltration (score 1). Epithelial degeneration was more pronounced (score 2) while sloughing scored one and ulceration remained absent (score 0). Fibrosis scored one indicating early connective tissue remodeling. These findings show partial prevention of tissue damage with noticeable reduction compared to the positive control, although complete protection was not achieved. The treatment group showed a stronger effect than the prophylactic group and approached the healing profile of the Omeprazole group. Minimal congestion and mucus depletion were observed (score 1), with complete absence of neutrophil infiltration (score 0) and lymphocyte infiltration (score 0). Epithelial degeneration was mild (score 1), while sloughing, ulceration, and fibrosis all scored zero. This pattern demonstrates marked enhancement of mucosal repair following ulcer induction and confirms the therapeutic potency of the extract. Overall, the histopathological scoring data collectively support that Zaghloul date extract is completely safe, provides partial prophylaxis, and exhibits strong therapeutic healing, closely approximating the effect of Omeprazole. These quantitative scoring outcomes are in full agreement with the qualitative histological observations.

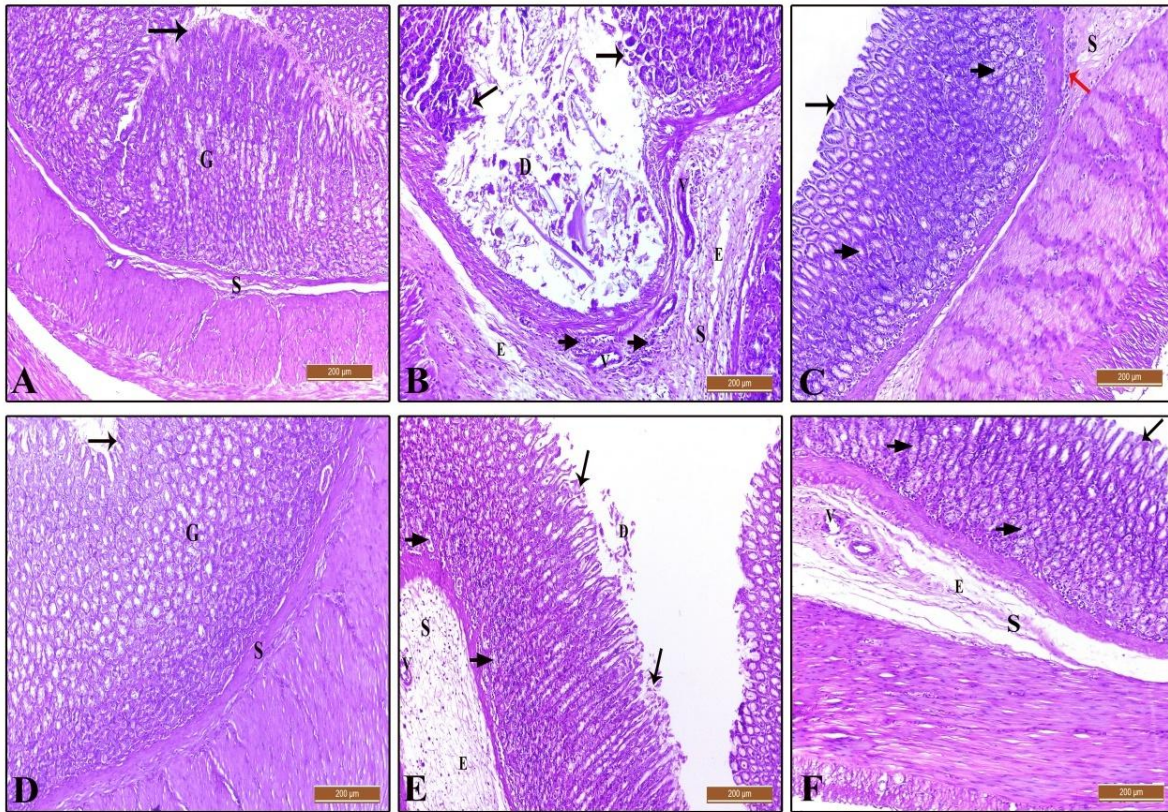


Fig (10): Photomicrograph of H & E stained gastric tissue sections of the experiment's groups (x100) showing: **A:** Control group showed the wall of the stomach appeared with normal orientation in which the gastric mucosa covered by normal columnar epithelium (arrow) as well as the gastric glands (G). The gastric mucosa rests on thin layer of gastric submucosa (S). **B:** Positive control (Gastric ulcer) group showed destroyed and sloughed gastric mucosa. The gastric epithelium and the gastric glands suffered from severe degeneration and destruction (arrow), sloughing (D) and ulceration. The submucosal layer (S) appeared very thick and engorged with highly dilated and congested blood vessels (V), severe edema (E) and aggregations of lymphocytes (arrowhead). **C:** Standard drug (Omeprazole) treated group showed more or less normal gastric wall with thick submucosal (S) layer containing lymphocytic infiltration (red arrow). the gastric mucosa composed of normal surface epithelium (arrow) and normal gastric glands (arrow head). **D:** Zaghloul date extract treated normal (Safety) group showed the wall of the stomach appeared with normal orientation in which the gastric mucosa covered by normal columnar epithelium (arrow) as well as the gastric glands (G). The gastric mucosa rests on thin layer of gastric submucosa (S). **E:** Zaghloul date extract prophylactic group showed the wall of the stomach appeared less normal with degenerated (arrow) and desquamated (D) gastric mucosa as well as degenerated gastric glands (arrow head). The gastric submucosa (S) appeared with edema (E) and dilated blood vessels (V). **F:** Zaghloul date extract treated group showed the wall of the stomach appeared with normal epithelium (arrow) as well as normal gastric glands (arrow head). The gastric submucosa (S) appeared with edema (E) and dilated blood vessels (V).

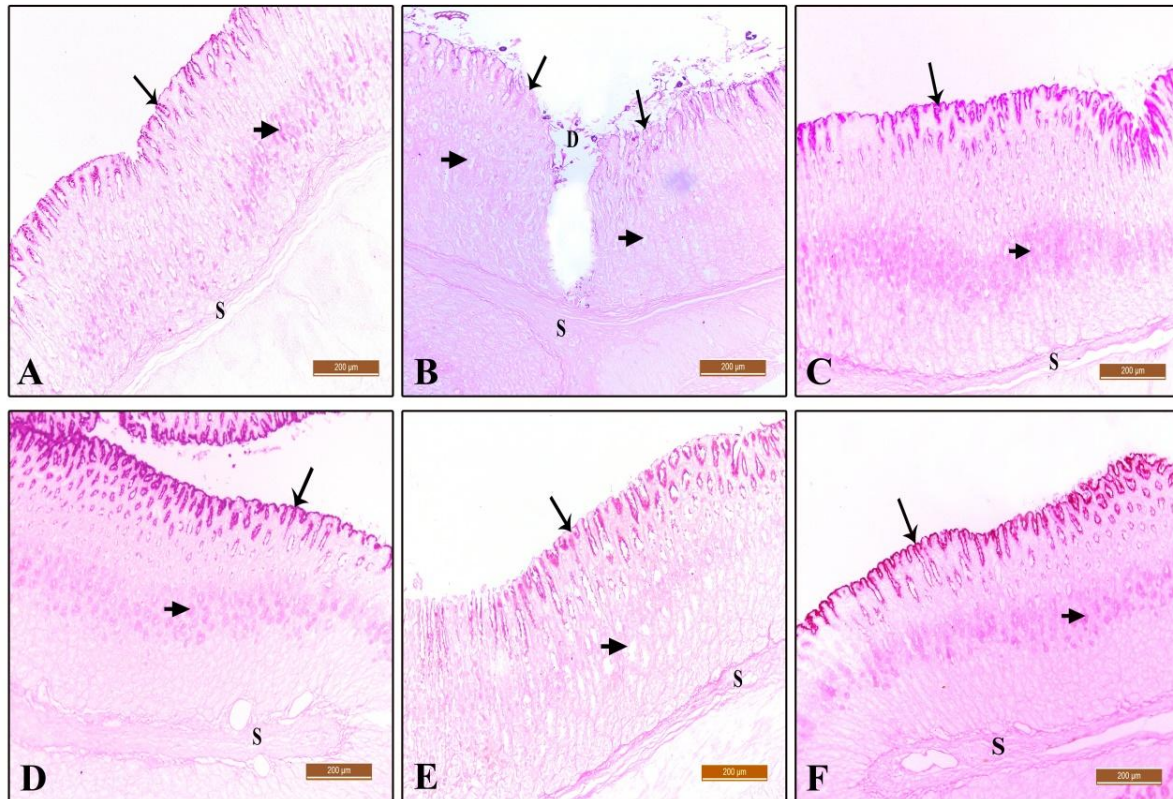


Fig (11):Photomicrograph of PAS stained gastric tissue sections of the experiment's groups (x100) showing:**A:**Control group showed the gastric mucosa showed a strong PAS reaction (arrow) and the gastric glands showed a moderate PAS reaction (arrowhead).The gastric submucosa (S) consisted of fibroelastic connective tissue showed weak PAS reaction.**B:**Ulcerative(Gastric ulcer) group showed highly destructed gastric mucosa (D) with destructed gastric epithelium (arrow) and gastric glands (arrowhead) showed negative PAS reaction. The gastric submucosa(S) appeared with weak PAS reaction.**C:**Standard drug (Omeprazole) treated group showed a strong PAS reaction in the gastric mucosa (arrow) while the gastric glands showed a moderate PAS reaction (arrowhead). The gastric submucosa (S) showed a weak PAS reaction.**D:**Zaghloul date extract treated normal (Safety) group showed a strong PAS reaction (arrow) and the gastric glands showed a moderate PAS reaction (arrowhead). The gastric submucosa (S) showed weak PAS reaction.**E:**Zaghloul date extract prophylactic groupshowed a moderate PAS reaction in the gastric surface epithelium (arrow) and the gastric glands showed a weak PAS reaction (arrowhead). The gastric submucosa (S) showed a moderate PAS reaction.**F:**Zaghloul date extract treated group showed a strong PAS reaction in the gastric surface epithelium (arrow) and the gastric glands showed a moderate PAS reaction (arrowhead). The gastric submucosa (S) showed weak PAS reaction.

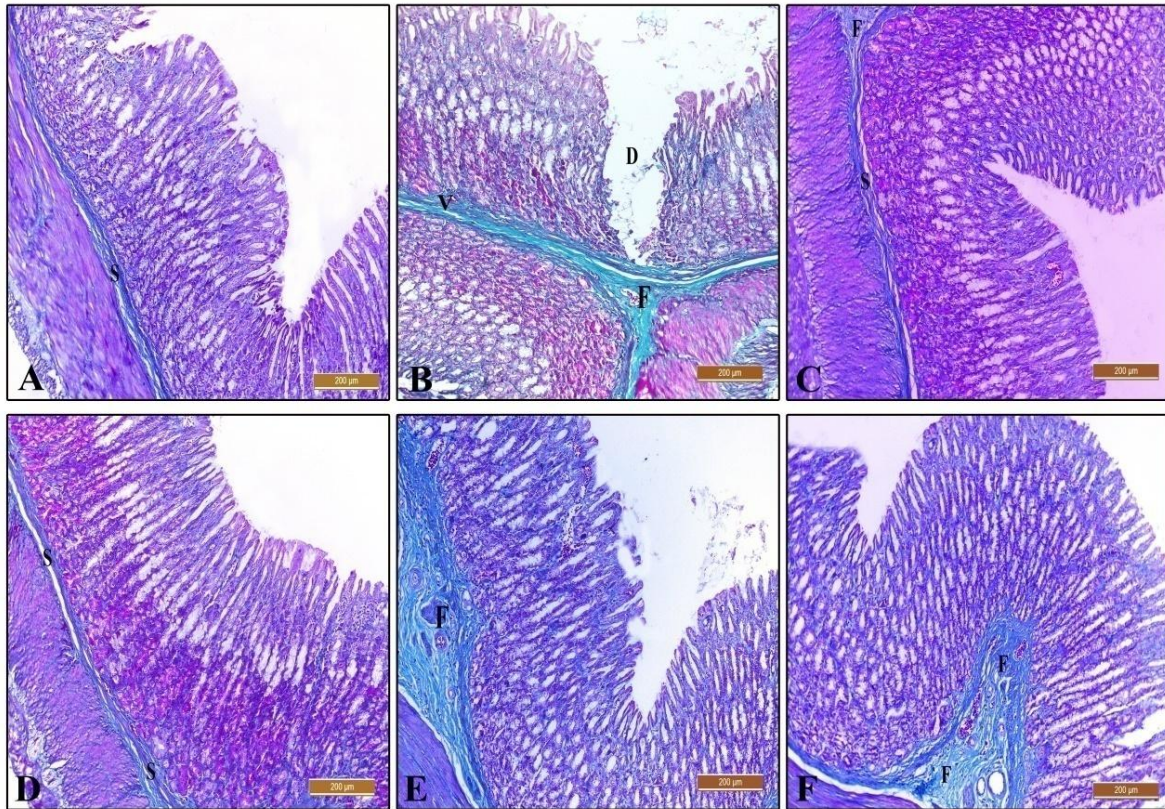


Fig (12): Photomicrograph of Masson's Trichrome stained gastric tissue sections of the experiment's groups (x100) showing, **A:** Control group showed normal gastric wall with normal the gastric epithelium and glands. The gastric submucosa(S) consisted of fibroelastic connective tissue containing numerous blood vessels. **B:** Ulcerativegroup showed highly destructed gastric epithelium and the gastric glands, sloughing and ulceration (D). The gastric submucosa appeared very thick (F) and engorged with highly congested blood vessels (V).**C:**Standard drug (Omeprazole) treated group showed more or less normal gastric wall with normalsubmucosallayer (S) with accumulated fibroelastic tissue (F).**D:**Zaghoul date extract treated normal (Safety) group showed normal gastric wall with normal the gastric epithelium and glands. The gastric submucosa (S) consisted of fibroelastic connective tissue containing numerous blood vessels.**E:**Zaghoul date extract prophylactic group showed normal gastric wall with very thick gastric submucosa (F)and engorged with highly congested blood vessels.**F:**Zaghoul date extract treated group showed normal gastric wall with very thick gastric submucosa (F) and engorged with highly congested blood vessels.

Table (3): Histopathological scoring of gastric lesions

Groups	Congestion of gastric vessels	Mucous depletion	Infiltration with Neutrophils	Infiltration with lymphocytes	Degeneration of the gastric epithelium	Sloughing of the gastric epithelium	Ulceration of the gastric mucosa	Fibrosis
Control group	0	0	0	0	0	0	0	0
Ulcerative group	4	3	2	3	3	3	3	3
Standard drug (Omeprazole) treated group	1	1	0	1	0	1	0	0
extract group	0	0	0	0	0	0	0	0
Protective group	1	2	1	1	2	1	0	1
Extract treated group	1	1	0	0	1	0	0	1

Histopathological scoring of gastric ulcer: 0= no change; 1< 25% tissue damage; 2= 26:50% tissue damage; 3= 51: 75% tissue damage; 4= 76: 100% tissue damage.

Discussion

Mucosal lining and bicarbonate make together the mucosal defense mechanisms of the stomach. Elevated levels of mucosal destructive factors including oxidative stress, inflammation and increased acid and pepsin secretion attribute to mucosal corrosion and subsequently GU formation ¹³.

GU increases by millions every year globally indicating low quality management of this issue ². Therefore, GU research rapidly develops focusing on the identification of new GU molecular mediators as well as new approaches for GU treatment and prevention ³.

A typical model of human acute GU can be induced in experimental animals by ethanol which is commonly used to assess potential therapeutic interventions ⁴⁹. Ethanol directly injures the gastric mucosa and prompts oxidative stress and inflammation ⁵⁰. Direct injury caused by ethanol includes gastric lesions through deep infiltration and dehydration of the gastric lining. Additionally, ethanol alters gastric blood flow and reduces the production of bicarbonate and mucin. Ethanol also causes indirect injuries by recruiting leukocytes, triggering gastric inflammation and increasing the oxidative stress leading to gastric tissue hemorrhage and necrosis ⁵¹. Oxidative stress is characterized by reduction of endogenous antioxidants including GSH and elevation of oxidation byproducts such as the lipid peroxidation product, MDA ⁵²⁻⁵⁴.

Cyclooxygenase (COX) enzymes are fundamental sentinels of the mucosal layer. They enhance the production of prostaglandins (PGs) which protect the stomach through the stimulation of mucus and bicarbonate secretion, maintenance of blood flow and enhancement of cell repair. Reduced levels of COX enzymes can lead to mucus erosion and subsequent GU formation¹³. Ethanol administration has been reported to impair gastric mucosal protection by suppressing prostaglandin production and reducing COX enzyme activity⁵⁵.

Inflammation is a basic leading cause of GU. The imbalance between the pro-inflammatory and anti-inflammatory cytokines orchestrates serial events that contribute to mucosal damage. The pro-inflammatory cytokine, TNF- α , is a key player in GU development. It sustains the inflammatory environment through upregulating other inflammatory mediators. Also, it induces apoptosis of the epithelial cells aggravating the erosion of the mucosal barrier⁵⁶. ICAM-1 contributes to leukocyte adhesion and infiltration into the ulcerated tissue aggravating the inflammatory responses⁵². Additionally, the downregulation of the anti-inflammatory cytokines such as IL-10 was considered a leading cause of GU development⁵⁶.

The present study investigated the gastroprotective and therapeutic effects of *Phoenix dactylifera* L. (Zaghloul cultivar) leaves extract against ethanol-induced gastric ulcer in rats through phytochemical, biochemical, molecular, and histopathological approaches.

The dose of the plant extract (400mg/kg) used in this study was selected following³⁶ as a safe non-toxic dose. In our experimental design, a confirmatory group (the extract group) was established by giving the rats the extract only. This group showed that the extract did not display any harmful or toxic effect on the rats. This was investigated by the oxidative stress, inflammatory biomarkers and gene expression levels that were statistically near to that of the control group. Also, the microscopic examination confirmed identical normal gastric structure with fully preserved mucosa and normal submucosal thickness.

In the present study, ethanol significantly caused GU damage as shown in the ulcerative group in which the levels of TAC and GSH were significantly reduced while the MDA level was significantly increased. Additionally, ICAM-1 and TNF- α were significantly elevated by 263.8% and 393.4%, respectively. COXI and COXII were significantly reduced by 40.7% and 42.5%, respectively compared to the control group. Histopathological examination revealed extensive mucosal destruction, epithelial and glandular degeneration, sloughing, ulceration and markedly thickened edematous submucosa infiltrated with lymphocytes and congested blood vessels, all of which confirming a successful model of GU. However, the level of IL-10 was significantly increased by 177.7% compared to the control group.

The results of the phytochemical analysis of the leaves extract in our study revealed high contents of phenolic and flavonoid compounds. These classes of secondary

metabolites are well known for their antioxidant and anti-inflammatory properties and have been widely reported to play a key role in protecting gastric mucosa against oxidative stress and inflammatory damage. Polyphenols and flavonoids have been widely reported to exert gastroprotective effects by enhancing antioxidant defenses, reducing inflammatory cytokines, and preserving gastric mucosal integrity in experimental ulcer models⁵⁷. The high phenolic and flavonoid contents detected in the present study are consistent with previous reports on date palm leaves and likely contribute to the observed biological activities³⁵.

Our findings showed that oral administration of the extract before ulcer induction significantly protected the mucosal layer from damage. This was investigated by the significantly higher levels of TAC and GSH and lower level of MDA in the protective group. ICAM-1 and TNF- α levels were significantly reduced while COXI and COXII were significantly elevated compared to the ulcerative group. Further histopathological examination showed lower degenerative changes in the mucosa and glands, submucosal edema and vascular dilation compared to the ulcerative group. However, the average level of IL-10 was significantly decreased compared to the ulcerative group.

Treatment of GU with the extract revealed its significant therapeutic roles against GU. The extract treated group showed significant increase in TAC and GSH mean levels and significant decrease in the average level of MDA. ICAM-1 and TNF- α were significantly reduced while COXI and COXII were significantly elevated compared to the ulcerative group. Histopathological examination showed significant restoration of the epithelial integrity and glandular architecture. The mucosal covering appeared markedly improved compared to the positive control. However, the mean level of IL-10 was significantly reduced compared to the ulcerative group.

These findings suggest that the extract effectively attenuates oxidative stress in accordance with other findings that have been reported for several phenolic-rich plant extracts that ameliorate ethanol-induced gastric lesions by restoring antioxidant defenses and suppressing oxidative damage⁵⁸. This antioxidant activity may be attributed to the free-radical scavenging capacity of phenolic and flavonoid constituents present in the extract, which are known to enhance endogenous antioxidant systems and protect gastric mucosal cells from ethanol-induced oxidative injury⁵⁹.

The reduction of TNF- α and ICAM-1 indicates a pronounced anti-inflammatory effect of the extract. The reduction in ICAM-1 suggests decreased leukocyte adhesion and inflammatory cell infiltration, whereas suppression of TNF- α reflects modulation of pro-inflammatory cytokine signaling. Similar anti-inflammatory effects have been reported for phenolic-rich plant extracts in experimental gastric ulcer models, and the observed activity in the present study was comparable to that of the reference drug Omeprazole^{60,61}.

The restored levels of COXI and COXII enzymes reveal fundamental roles of the extract in the enhancement of the gastric mucosal protection and healing through remodeling the COXI and COXII enzymes pathways. Similar findings have been reported in experimental gastric ulcer models where natural compounds restored COX expression and prostaglandin synthesis, thereby improving mucosal defense ⁶².

Partially disagree with our results, many researches focused on the alteration of the inflammatory markers during GU induction and treatment showed that GU increases the levels of the pro-inflammatory cytokine TNF- α and lowers the levels of the anti-inflammatory cytokine IL-10. Whereas GU treatment was found to decrease TNF- α and elevate IL-10 ⁶³⁻⁶⁶.

On the other hand, compliant with our findings **Gilani et al.** ⁶⁷ and **Elbadr et al.** ⁶⁸ investigated that both TNF- α and IL-10 were elevated due to GU induction and were reduced by GU treatment. Additionally, IL-10 has been proposed to be a predictor of the severity or mortality associated with acute or post-acute CoV-2 infection. Thereby, IL-10 was considered as an endogenous danger signal that is released by tissues undergoing damage to protect the body from harmful extra-inflammation ⁶⁹. Also, the pro-inflammatory cytokines including TNF- α were reported to be released due to the activation of the M1 macrophages contributing to gastric ulceration, while the anti-inflammatory cytokines such as IL-10 are produced by the M2 macrophages to diminish the severity of GU associated inflammation ¹². These findings collectively suggest that IL-10 is a first aid in the anti-inflammatory strategies displayed by the body in response to the inflammatory disorders.

Moreover, TNF- α / IL-10 ratio was considered to serve as a more precise and informative index of pro- to anti-inflammatory immune homeostasis, a fundamental process in protecting the body from nonfunctional chronic inflammations ⁷⁰. Several studies have investigated that the TNF- α / IL-10 ratio may reveal the extent of severity of many inflammatory disorders as well as the degree of successful of a specific treatment. A Russian study on children suffering cystic fibrosis has revealed that the live rubella vaccine declined the TNF- α /IL-10 ratio ⁷¹. **Goswami et al.** ⁷² observed an increase of the TNF- α /IL-10 ratio in the blood of acute myocardialinfarction patients. **Kumari et al.** ⁷³ have reported that the elevated TNF- α /IL-10 ratio may be a fundamental cause of the progression of coronary artery disease. A recent study released by **Al-Zubaidi et al.** ⁷⁴ has concluded that the TNF- α /IL-10 ratio may be efficiently used to determine the severity of tuberculosis and may also be a good biomarker for the evaluation of the early response to treatment.

Consistently, our study showed that TNF- α /IL-10 ratio was equal to 3.7 in the control group and it was significantly increased to 8.1 in the ulcerative group. Supportive to the previously mentioned conclusion of **Al-Zubaidi et al.** ⁷⁴, the decreases to 6.8, 6.8 and 6.7 values exhibited by the protective, extract and OM treated groups, respectively compared to the ulcerative group may confirm an early anti-inflammatory response triggered during the period of our treatment (7 days). This finding may confirm that

using the ratio between TNF- α and IL-10 is more reliable in determining the inflammatory state progression than using the absolute value of either of them alone. Therefore, the elevated TNF- α /IL-10 ratio may serve as an outstanding indicator for the progression of chronic inflammation and associated disorders such as gastric cancer.

Many proteins such as VIM, RAD21, E2F1 and NIPBL were reported to be involved in fundamental cell cycle activities and their dysregulation has been implicated in the progression of various diseases including intestinal diseases and gastric cancer (GC). To the best of our knowledge, our study is the first attempt to investigate the roles of these proteins in GU induction and/or healing.

VIM is a key type III intermediate filament protein found in cells of mesenchymal origin such as macrophages, fibroblasts, endothelium and vascular smooth muscle cells. It plays fundamental roles in retaining cell shape, cytoplasm integrity as well as wound healing^{20,75,76}. However, it has been found to be implicated in numerous pathological conditions involving inflammatory, intestinal, infectious, vascular diseases as well as malignancies and diseases characterized by fibrosis⁷⁶.

Inflammation which is a hallmark of ulceration was reported to trigger disassembly of the intracellular VIM filaments via post-translational modifications. The modified VIM oligomers accept new antigenic and immunogenic properties as well as extra affinity to be secreted to the extracellular space as ECV⁷⁷. Recently, ECV was found to be associated with many biological processes involving cell signaling, tissue repair and immune responses. ECV acts as a damage-associated molecular pattern protein (DAMP) that modulates the immune system functions¹⁸. The elevated levels of ECV were reported to disrupt the structure and function of tissues in diseases like inflammatory and neoplastic diseases. ECV was also reported to trigger macrophages to activate NF- κ B leading to the release of pro-inflammatory cytokines such as TNF- α exacerbating the inflammation and causing tissue damage^{18,77}.

Furthermore, TNF- α has been reported to stimulate the secretion of VIM by macrophages creating a cycle that aggravate the inflammatory response⁷⁸. Also, VIM was reported to influence the neutrophil functions being a new molecular target for therapeutic approaches that aim to regulate the neutrophilic activity in different pathological conditions¹⁸. Additionally, **Håversen et al.**⁷⁹ have investigated that VIM plays a principal role in regulating macrophages inflammatory activities during atherogenesis. Also, VIM expression was suggested to be a significant biomarker for poor prognosis in GC⁷⁵.

In accordance, our study showed that VIM was up-regulated in response to ethanol induction of GU. Here, the ulcerative group displayed highly significant increase in VIM compared to the control group. The protective and OM treated groups showed highly significant reduction of VIM level while the extract treated group showed lower effect with significant reduction of VIM level compared to the ulcerative group. Therefore, our study suggests VIM down regulation as a promising approach for ulcer

management. Furthermore, histopathological investigations in our study revealed that the protective and treated groups displayed marked reduction in neutrophils, macrophage and lymphocytes infiltration along with significant reduction in fibrosis suggesting VIM as a fundamental mediator of the major changes associated with ulceration.

Recent researches focused on the E2F1, RAD21 and NIPBL proteins revealed their key roles in fundamental cellular activities such as DNA duplication, mitotic division, regulating expression of many genes including genes involved in DNA damage repair, chromatin assembly and condensation, chromosome separation and checkpoint control.

E2F1 is the principal member of the Early 2 factor (E2F) family that modulates the cell cycle through various actions including the enhancement of DNA replication and repair²¹. This family has been known to have significant roles in the detection and repair of damaged DNA, cell proliferation and apoptosis. Additionally, E2F1 was reported as a principal modulator of metabolic activities in normal as well as pathological conditions^{22,80}.

In the present study, the average expression level of E2F1 gene was increased significantly by 154% in ulcer-induced rats compared to the control group. The extract treated group showed no significant alteration compared to the ulcerative group. On the other hand, rats protected by the plant extract before ulcer induction and those treated with Omeprazole revealed significant increase in the expression levels of E2F1 gene compared to the ulcerative group.

The elevated expression of E2F1 in the ulcerative group comes in accordance to **Stevens and Thangue**²² who reported E2F1 to be up regulated in response to DNA damage.

The further increase in E2F1 in the protective and OM groups along with the unchanged expression in the extract treated group compared to the ulcerative rats may indicate that E2F1 is produced for damaged DNA repair and tissue regeneration through indirect or multistep pathway that involves one or more molecules that have been protected by the extract before GU induction and reserved by OM treatment but could not be reserved by the extract treatment. According to **Lin et al.**⁸¹, E2F1 was reported to be selectively induced in response to DNA damage in a process mediated by ATM-dependent phosphorylation. The ATM/ATR and Chk2 kinases dependent phosphorylation of E2F-1 was reported to stabilize E2F1 and prevent its normal turnover. Another study released by **Stevens and Thangue**²² has investigated the activation of E2F1 in response to DNA damage via checkpoint kinase. Therefore, it may be suggested that the earlier administration of the extract prevented an inhibitory or degradation pathway of the ATM/ATR and Chk2 kinases and Omeprazole treatment reserved these molecules preventing E2F1 turnover and enhanced its production leading to its exceeded

expression levels in both the protective and Omeprazole treated groups. However, further studies are needed to clarify more details about the exact anti-ulcer mechanism of E2F1 and how it can be modulated for efficient anti-ulcer approach.

Similarly, RAD21 and NIPBL showed significant increase in the ulcerative group. Extra induction of RAD21 and NIPBL was shown in the protective and OM treated groups, the extract treated group also exhibited further increase in RAD21 and NIPBL levels but its effect was less than that of the protective and OM treated groups.

RAD21 is a component of the cohesin complex which is essential for the cohesion of sister chromatids after DNA replication⁸². Additionally, RAD21 was known to be involved in DNA damage repair and inflammation modulation. **Deardorff et al.**⁸³ have investigated that mutations in RAD21 lead to extreme defects in functions and worsen cognitive and structural clinical findings due to the impairment of DNA damage response and disruption of transcription. According to **Murrell and Gibson**⁸⁴, RAD21 can enhance inflammatory response, DNA repair and cell division contributing to regeneration of the damaged epithelia of the mucosal layer. Furthermore, it was found that Caspase cleaves RAD21 producing a pro-apoptotic carboxyl-terminal product that augments the cell death signal⁸⁵.

The sister chromatid cohesion 2 (SCC2) human homolog (NIPBL) is a cohesin loading factor that functions mainly in the deposition of cohesin onto the sister chromatids supporting their cohesion. Additionally, NIPBL was investigated to be involved in DNA repair, cell apoptosis, regulation of gene expression, cell cycle and development. Also, NIPBL mutation was identified in the Cornelia de Lange syndrome (CdLS), a developmental disorder characterized by many abnormalities including gastrointestinal abnormalities^{25,28}

collectively, these findings suggest that RAD21 and NIPBL are pleiotropic proteins produced at first for inflammation modulation, and after returning immune homeostasis contributes to the regeneration of the damaged tissues through enhancement of apoptosis of severely damaged cells and thereby decreasing inflammatory stimulants (DAMPs) and associated extra inflammatory responses, then increases epithelial cell proliferation and subsequently DNA replication which requires augmented levels of RAD21 and NIPBL for sister chromatid cohesion after DNA replication. Therefore, the extra increase in RAD21 and NIPBL due to our treatments may reflect successful return to homeostasis through decreasing the oxidative stress and inflammation as well as the enhancement of cell proliferation and tissue regeneration.

Histopathological examination confirmed the previously mentioned biochemical and molecular findings. Severe mucosal destruction, epithelial degeneration and inflammatory infiltration are hallmark features of ethanol-induced gastric ulcer models and have been consistently reported in the literature⁸⁶. Treatment with plant extracts rich in phenolics and flavonoids has been shown to ameliorate these histological changes by promoting restoration of mucosal architecture, reducing inflammatory cell

infiltration and preserving epithelial integrity. In the present study, Zaghloul leaves extract markedly improved gastric architecture, enhanced mucin production as evidenced by PAS staining and reduced fibrosis in treated animals demonstrating its capacity to promote gastric mucosal healing and preserve tissue structure. These histological observations align with similar reports where antioxidant and anti-inflammatory phytochemicals attenuated ethanol-induced gastric injury and facilitated tissue repair⁸⁷.

Overall, the gastroprotective and therapeutic effects of *Phoenix dactylifera* leaves extract can be attributed to its antioxidant, anti-inflammatory and mucosal protective properties which collectively contribute to attenuation of oxidative damage, modulation of inflammatory mediators and enhancement of tissue repair mechanisms^{35,88}.

Conclusion

The present study suggests that Vimentin plays fundamental roles in the development of gastric ulcer enhancing the associated inflammatory, oxidative stress as well as the histological changes. E2F1, RAD21 and NIPBL are suggested to be produced in response to DNA and tissue damage triggered by GU induction to induce the regenerative process which was supported and enhanced by the extract and the standard drug which led to extra production of these proteins. *Phoenix dactylifera* (Zaghloul cultivar) leaves extract exhibited significant gastroprotective and therapeutic activities against ethanol-induced gastric ulceration. The extract exhibited notable antioxidant effects by restoring total antioxidant capacity and glutathione levels while reducing lipid peroxidation. In addition, it showed pronounced anti-inflammatory activity through the suppression of inflammatory mediators such as TNF- α and ICAM-1, reduction of TNF- α /IL-10 ratio and the restoration of cyclooxygenase enzymes involved in gastric mucosal defense. Gene expression analysis further indicated that the extract modulated stress- and regeneration-related genes including **E2F1, RAD21, NIPBL and Vimentin**, suggesting attenuation of epithelial injury and inflammatory tissue damage. Histopathological evaluation confirmed these findings by demonstrating improved gastric architecture, enhanced mucin production and reduced tissue damage in treated animals.

Further studies are recommended to isolate the active constituents and explore the underlying molecular mechanisms and clinical applicability.

Ethical Approval: The study is reported in accordance with ARRIVE guidelines. Anesthetic procedures, handling of the animals, and their termination were compiled with the ethical guidelines set by the Medical Ethical Committee of the National Research Centre, Egypt.

Consent to participate: The research does not involve human subjects.

Consent to publish: There are no conflicting interests that could have influenced the research results presented in this paper.

Clinical trial: Not applicable.

Author contributions SE, AME & MME conducted the phytochemical characterization, interpreted the results, and assisted in the preparation of the manuscript. HFA, HSK, WK, KAH, MBS & MIM conducted the biological investigations, performed the statistical analysis of the collected data, interpreted the results and prepared the manuscript. MSS performed and wrote the results of the histopathological analysis. All authors wrote, reviewed and approved the final version of the manuscript.

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