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THE POTENTIAL PROTECTIVE EFFECTS OF ELLAGIC ACID AND COENZYME Q10 AGAINST EXPERIMENTALLY-INDUCED GASTRIC ULCER IN RATS

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ABSTRACT
Gastric ulcer is a persistent gastrointestinal disease; it involves 10% of the world's population and can lead to bleeding and death. The presented study was conducted to spotlight the leverage of Ellagic (EA) acid and Coenzyme Q10 (CoQ10) as gastro-protective agents against gastric ulcers in rat models, and we aimed to focus on gaining a better understanding of the effects and the mechanistic pathways of action of EA and CoQ10. Male rats were distributed to seven groups: group I normal vehicle normal control, group II indomethacin (INDO) ulcer control, and pre-treatment groups that include; group III ranitidine (RA), group IV EA, group V EA + RA, group VI CoQ10, and group VII CoQ10 + RA. For 10 days before induction of the ulcer with INDO, each rat received either vehicle (carboxymethyl cellulose +1% tween 80) or other doses intragastrically, and on day 10, they received INDO. After 6 hours of INDO treatment, rats were sacrificed, and their stomachs were excised to evaluate histopathological changes, gastric acidity, inflammatory molecules, oxidative stress, and apoptotic mediators. Results showed that a single 100mg/kg dose of INDO significantly elevated gastric acidity, expression of caspase-3, malondialdehyde (MDA), and gastric tumor necrosis factor-alpha (TNF-α), and significantly diminished levels of reduced glutathione (GSH), nitric oxide (NO), and superoxide dismutase (SOD) activity compared to normal control. Pre-treatment with EA and CoQ10, each alone and in combination with RA, preserved the histopathological changes and gastric acidity within the normal limits and produced significant raises in GSH, SOD, NO levels, and significant decline in MDA, TNF-α gastric levels, as well as a decrease in caspase-3 up-regulation, compared to ulcer control values.

1. INTRODUCTION

Gastric ulcer is a persistent gastrointestinal disease; it involves 10% of the world population; it is described as a disruption of gastric mucosal integrity[1]; this occurs due to impairment in the balance between the defensive and destructive factors in the gastrointestinal tract.[2] These factors embrace both endogenous and exogenous factors.[3] Aggressive endogenous factors like acid, stress, active free radicals, TNF-α, leukotrienes, bile reflux, reactive oxygen species (ROS), and proteolytic enzymes. Aggressive exogenous factors like non-steroidal anti-inflammatory drug (NSAID), H. pylori bacterial infection, ethanol consumption, and cigarette smoking.[4] In contrast, defensive factors like prostaglandins PGE2 and PGI2, NO, mucosal blood flow, mucus, bicarbonate, enzymatic antioxidants like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), and non-enzymatic antioxidants like glutathione (GSH) work as a barrier against gastric damages.[5] NSAID drugs are broadly used to treat inflammatory pain and majorly cause gastric ulcers and bleeding. Via suppression of cyclo-oxygenase enzymes, NSAIDs cause lowering in prostaglandins, NO, mucus, bicarbonate, and mucosal blood flow, and generation of ROS, and formation of free radicals, which all contribute to gastric mucosal injury and bleeding.[6] Other NSAID-induced gastric lesions and ulcer mechanisms embrace the increased mucosal expression of pro-inflammatory molecules like TNF-α and apoptotic molecules like caspase-3.[7]

Tumor necrosis factor-α (TNF-α) is an inflammatory cytokine that is embroiled in the pathogenesis of gastric ulcers; it can induce apoptosis of gastric parietal cells[8], and act as an inhibitor of constitutive nitric oxide synthase (cNOS), leads to disturbance in the production of NO in gastric mucosa[9], after that lead to depletion of mucosal blood flow, increased lipid peroxidation, increased production of ROS, and generation of free radicals.[10]

Indomethacin is an NSAID proven to induce gastric lesions and can potentially cause erosions and damage to the gastrointestinal mucosa, induces ulcer, and exacerbates the previously existing gastric ulcer. Hence, it is apparent that INDO-induced gastric lesions are multi-causal disorders embracing reduction in prostaglandins, free radical formation, raised production of TNF-α, and disturbance of NO production in gastric tissues. Moreover, therefore, using compounds with antioxidant activity and the capability to control neutrophil infiltration and regulate NO levels may be valuable in protecting against INDO-induced gastric ulceration.[11]

New therapeutic researches for treating gastric ulcers aim to minimize the effect of aggressive and potentiating defense factors. However, these treatments are not perfectly effective and produce mild to serious adverse effects, particularly when used for a long period.
Although gastric acid secretion is a major component of this gastric ulcer ailment, its control was the main target for a cure for decades. H2 blockers (such as ranitidine) or proton pump inhibitors (PPIs) (such as omeprazole) were used.[12] Overall, PPI is regarded to have more advantageous restrained effects on acid secretion than H2 blockers. These treatments have significant drawbacks because they cause many adverse effects.[13]

Ellagic acid (EA) is a polyphenolic plant that occurs naturally, found in some fruits and vegetables like pomegranates, cranberries, raspberries, strawberries, and walnuts.[14] It has antioxidant, anti-inflammatory, and anti-carcinogenic pharmacological properties.[15,16] EA has an effective scavenging influence on hydroxyl anion, oxygen anion, and superoxide anion[17]; it causes fracturing of radical chain reactions, metal chelation, and TNF-α attenuation; EA can also increase the levels of NO and GSH and activate antioxidant-like GPX, CAT, and SOD enzymes.[18] EA elicited a bactericidal effect against H. pylori, which is among the causative agents of gastric ulcers[19] and provides significant protection by the suppression of lipid peroxidation and myeloperoxidase (MPO) activity.[20] EA can also decrease neutrophil infiltration and increase PGE2 by increasing the expression of COX enzymes.[21]

All of these factors helped retain the balance between pro-inflammatory/anti-inflammatory mediators via up-regulation of mucosal growth factor levels to prevent and accelerate ulcer healing.

Coenzyme Q10 (CoQ10) is a substance with antioxidant and anti-inflammatory activity[22], produced in the body, and is also obtained from the diet, particularly by meat, also obtained from fish, vegetable oil, poultry, and nuts.[23] Cardiovascular diseases, aging, cancer, neurodegenerative diseases, diabetes, and hypocholesterolemic statin drugs decrease CoQ10 content. More than CoQ10 in the meal is needed to increase its level. A significant increase of CoQ10 in serum requires the administration of about 100mg of CoQ10/day.[24]

CoQ10 is a powerful free radical scavenger and neutralizer; CoQ10 possesses an antioxidant influence on cell membranes and mitochondria and prevents lipids oxidation; this effect is related to its ability to neutralize free radicals and to replenish antioxidants like endogenous GSH-PX, GSH, and SOD, COQ10 also has anti-diabetic properties.[25] CoQ10 has a protective role against cancer, aging, neurodegenerative disorders, cardiovascular impairments, and diabetes mellitus is well established[26,27], CoQ10 also has an anti-apoptotic activity that mediates its powerful activity.[28]

The current study performed to spotlight the functions of EA and CoQ10 as a gastro-protective agent (pre-exposure prophylactic) against INDO-induced gastric ulcer in rat models, and we aimed to get a better knowledge of the effects and mechanistic pathways of action of EA acid and CoQ10.

2. MATERIALS AND METHODS

2.1. Drugs

Indomethacin (INDO) was gifted by KPC Industries Co. (Giza, Egypt). Ranitidine (RA) was obtained from Nile Co. (Cairo, Egypt) for pharmaceutical industries. EA was purchased from pure bulk laboratories (NY, USA). CoQ10 was purchased from bulk supplement laboratories (Henderson, NV, USA), and Tween 80 was manufactured by oxford lab chem (Mumbai, India).
All other used chemical substances were of excellent quality and chemical analytical grade.

2.2. Animals

Adult Males albino rats (180–210 gm), aged Ten weeks, were purchased from the Faculty of Veterinary Medicine, Tanta, Egypt.

The rats were kept in wire cages in the animal care unit at the Faculty of Pharmacy, Tanta University, Tanta, Egypt, at pathogen-free conditions with equal light: dark cycle, and maintained at 25 ± 2 C. and All rats had *ad libitum* access to proper chow and tap water. All rats were acclimatized for 1 week before the experiments. After this period, all rats fasted for 24h before experiments; then, the rats were weighed and allocated randomly into seven experimental groups, each Group consisting of 16 rats. Rats in Group I were designated normal Control group, rats in Group II were the INDO-induced ulcer group; Group III was RA + INDO group; Group IV was EA + INDO group; Group V was EA + RA + INDO group; Group VI was the CoQ10 group, Group VII was CoQ10 + RA + INDO group.

This study was performed in conformity with guidelines for using laboratory animals and approved by the Research Ethics Committee of the Faculty of Pharmacy, Tanta University (Tanta, Egypt).
INDO was given for ulcer induction by oral gavage at a dose of $100 mg.kg^{-1}$.[29]

Rats in the control group received the suspending vehicle for INDO, RA, EA, and CoQ10 (Carboxymethyl cellulose + 1% Tween 80).

For rats of pre-treatment groups, rats in group III Ranitidine (RA) was given at a dose (of $100 mg.kg^{-1}.day^{-1}$) [30], or EA at a dose ($75 mg.kg^{-1}.day^{-1}$) for group IV[31], or EA at a dose ($75 mg.kg^{-1}.day^{-1}$) + RA at a dose ($100 mg.kg^{-1}.day^{-1}$) for group V, or CoQ10 at a dose ($100 mg.kg^{-1}.day^{-1}$) for group VI[32], or CoQ10 at a dose ($100 mg.kg^{-1}.day^{-1}$) + RA at a dose ($100 mg.kg^{-1}.day^{-1}$) for Group VII were given intragastrically by (gavage) for 10 sequential days.

On the 10th day, these rats fasted for 24 hrs to ensure that their stomachs were empty except for water, which was prevented for 2 hrs before ulcer induction with INDO.[33] Then INDO was administered intragastrically 1 hr after the ranitidine or other doses. Rats in Groups I and II were administered vehicle instead of other drugs for 10 consecutive days administrated vehicle or INDO, respectively. After six hours of the INDO or vehicle oral gavage, the rats were euthanized by ether asphyxiation[34], and their stomachs were quickly excised and processed for histopathological, immuno-histochemical examination and evaluation of gastric damage.

2.3. Measurement of gastric pH

On day 10, the experiment terminated, and the rats were killed. Their stomachs were excised, squeezed, and drained into centrifuge tubes[35], then centrifugation of the collected content at 1000 rpm, 4 Cº for 10 min, and the gastric juice collected from the clear supernatant.

Gastric pH measurement was performed according to the method of[36], using a pH meter, a Model HI 110 pH meter (Hanna Instruments, Providence, RI).

2.4. Stomach tissues Preparation

After collecting gastric juice, all stomachs opened along the greater curvature and were washed with cold saline. 8 of the 16 stomachs of each group were immediately fixed in buffered formalin (10%) for histological and immuno-histological examinations, and the other 8 stomachs were cut into pieces, weighed, and stored at -80 Cº for biochemical studies. 100 mg of the stomach was homogenized in 1 ml of cold PBS using the Polytron PT 3100 homogenizer; the output was then centrifuged at 3000 rpm,4 Cº for 20 min, and the clear resultant supernatant isolated was used for further assessment of gastric contents of GSH, lipid peroxides (measured as malondialdehyde MDA), NO, SOD and TNF-α.

2.5. Determination of reduced glutathione

Determination of GSH content in the homogenate of a tissue sample of the stomach was performed according to the method of Ellman. Which based on the reductive cleavage of Ellman's reagent {(bis-3-carboxy-4-nitrophenyl) disulfide} with SH group of reduced glutathione to produce a yellow color product which measured at 412nm in a spectrophotometer. Values of the GSH concentration in gastric tissue homogenate are calculated as µmol/gm starting tissue using a standard curve.[37]

2.6. Determination of malondialdehyde

MDA gastric content was determined using a colorimetric MDA diagnostic assay kit obtained from Biodiagnostics (Cairo, Egypt). Accordingly, the principle of the method, thiobarbituric acid (TBA), will interact with MDA existent in a homogenate tissue sample to formulate the TBA-Reactive substrate. Then the absorbance of the resultant pink-colored products was measured using a kit-provided formula, the values were calculated and expressed as nmol of MDA/gm tissue.[38]

2.7. Determination of NO

The gastric content of NO was determined using a colorimetric nitric oxide assay kit obtained from Biodiagnostics (Cairo, Egypt). Accordingly, the principle of the method, in the acidic medium in the existence of nitrite, the created nitrous acid diazotizes sulphanilamide, and the output coupled with N-(1-naphthyl) ethylene-diamine, and the resultant bright reddish-purple color product measured using spectrophotometer at 540 nm using a kit-provided formula, the nitrite values in samples were calculated and expressed as µmol NO/ gm tissue.[39]

2.8. Determination of superoxide dismutase enzyme activity

The gastric content of SOD was colorimetrically determined using a SOD assay kit obtained from Biodiagnostics (Cairo, Egypt). The principle is based on the potency of the SOD enzyme to attenuate the reduction of nitrite tetrazolium (NBT) dye by phenazine methosulphate (PMS). The absorbance was recorded at 560nm in a spectrophotometer for 5 min. The values of SOD activity were calculated using the formulas attached to the kit and presented as u SOD/gm tissue.[40]

2.9. Determination of tumour necrosis factor-α

Gastric TNF-α contents were determined according to the protocol of the manufacturer using Rats TNF-α ELISA kit, which was obtained from Shanghai Sunred Bio (SRB) Technology Co, Ltd (CHINA). The sensitivity of the kit was 5ng TNF-α/L, and using a microplate reader LMR 9602 (labnics equipment

USA), the absorbance was measured at 450nm. The intensity of the resultant colored product is positively correlated to the content concentration of Rat TNF-α existent in the sample.

2.10. Histopathological evaluation

The excised stomach sections were fixed for 24hrs in buffered formalin (10%), then immersed within paraffin to create blocks. The samples were then subsequently sectioned (3-µm thickness) using a microtome RM-2135 (Leica, Germany); the sections were mounted on slides and stained using hematoxylin and eosin (H&E) solution. Then the materials were unthinkingly evaluated by a board-certified pathologist under a light microscope.

2.11. Immuno-histochemical evaluation

The staining was performed according to.[41] The wax of the serial sections was removed, hydrated, and embedded in an antigen retrieval (EDTA solution, PH 8), then treated with 0.3% H_2O_2 and protein block, then incubated with primary caspase-3 antibodies (R&D et al., Minneapolis, Minn, USA; 1:100 dilution). The slides were rinsed thrice with PBS, then incubated with anti-mouse IgG secondary antibodies (EnVision + System HRP; Dako) for 30 minutes at 25 Co, and were visualized using diaminobenzidine commercial kits (Liquid et al.; Dako) and counterstained with Mayer's hematoxylin. As a negative control procedure, the primary antibody was replaced by normal mouse serum. The labeling index of caspase-3 was expressed as the percentage of positive cells per 1000 counted cells in about 8 to 10 high-power fields.

2.12. Statistical analysis

Data Analysis was performed with GraphPad Prism (version 7.0). All data for PH, GSH, MDA, NO, SOD, and TNF-α determinations were expressed as a mean of 8 animals ± SD, and data for caspase-3 was expressed as the mean of 2 animals ± SD. Statistical comparisons between means of groups were performed using an ordinary one-way analysis of variance (ANOVA) followed by Tukey's multiple tests for comparisons. Statistical significance was considered at p-value ≤ 0.05.

3. RESULT

3.1. Effect on pH

Indomethacin alone administration produced a significant decline in the pH of gastric juice, and the change was by 30.4% relative to the pH value of gastric juice from rats in Groups I normal control (Table1). pH values dropped from 2.92 ± 0.29 to 2.03 ± 0.10.

Table 1: Effects of pre-treatment of EA and CoQ10 each alone and in combination with RA on gastric pH, GSH, and MDA.

GROUP \ PARAMETER N=8/group	pH	GSH µmol/gm	MDA nmol/gm
CONTROL	2.92±0.29	215.46±18.31	66.80±10.57
INDO	2.03±0.10*	93.29±10.15*	126.07±9.88*
RA	3.20±0.19^a	185.96±19.57*^{,a}	79.50±9.00^a
EA	3.16±0.07^a	178.93±24.83*^{,a}	83.02±7.88*^{,a}
EA+RA	3.40±0.21*^{,a}	204.86±25.77^a	77.43±8.46^a
CoQ10	3.11±0.14^a	197.17±11.13^a	84.60±11.54*^{,a}
CoQ10+RA	3.37±0.23*^{,a}	213.03±10.34^a	82.54±7.34*^{,a}

*****p ≤ 0.05, significant from normal control (I) group.
^{a}p ≤ 0.05 significant from ulcer control (INDO-induced ulcer) group (II).
^{b}p ≤ 0.05 significant from RA + INDO group (III).
^{c}p ≤ 0.05 significant from EA group (IV).

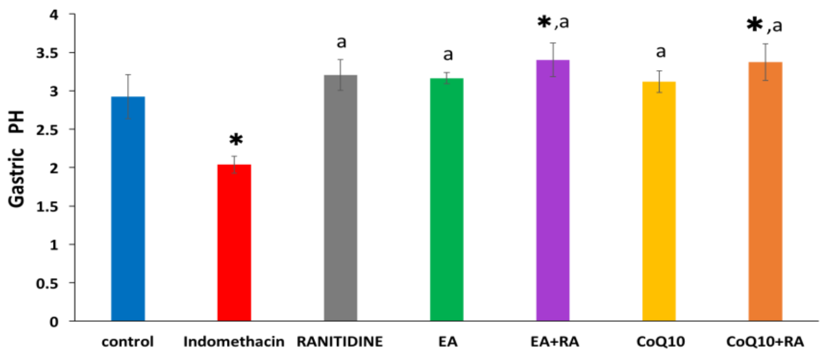

Figure 1: Effects of pre-treatment of EA and CoQ10 each alone and in combination with RA on gastric pH. Data are expressed as mean (± SD) (n= 8 rats). Values are significantly different from normal* or INDOa alone (ulcer control a) (p-value ≤ 0.05), n=8.

Pre-treatment with RA, EA, EA+RA, COQ10, and COQ10+RA separately significantly increased pH values by 36.5%, 35.7%, 34.7%, 40.4%, 39.7%, respectively than that in the ulcer group rats that received INDO alone and significantly preserved the pH values at higher levels than those of normal control (Figure 1).

3.2. Effect on gastric GSH

INDO administration in rats of the ulcer group significantly (p-value ≤ 0.05) caused a decrease in the levels of stomach content of reduced GSH relative to GSH levels in gastric tissues of the normal rat group by 56.7%. (Table 1).

Pre-treatment with RA, EA, EA+RA, COQ10, and COQ10+RA each alone significantly increased GSH by 49.8%, 47.8%, 54.4%, 52.65%, 56.25%, respectively, relative to the ulcer group that received INDO alone, also RA and EA pre-treatment administration had caused a significantly different effect compared to GSH levels in the normal control group. In contrast, EA+RA, COQ10, and COQ10+RA administration had no significant difference from that in the normal group (Figure 2).

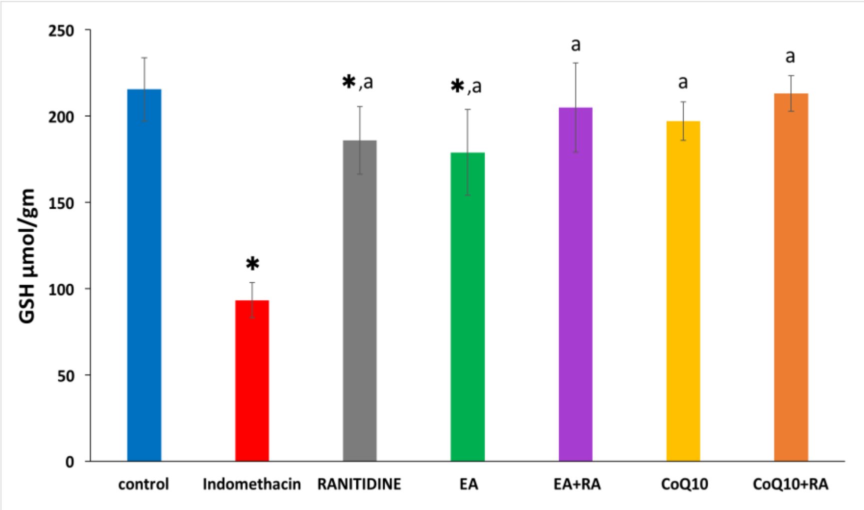

Figure 2: Effects of pre-treatment of EA and CoQ10 each alone and in combination with RA on gastric GSH content. Data are expressed as mean (± SD) (n= 8 rats). Values are significantly different from control* or from INDO[a] only (ulcer) (p-value ≤ 0.05).

3.3. Effect on gastric MDA

INDO administration in rats resulted in a significant increase (p-value ≤ 0.05) in gastric tissue MDA content levels relative to MDA levels in the normal control rats group by 47% (Table 1).

Pre-treatments with RA, EA, EA+RA, COQ10, and COQ10+RA separately produced significant decreases in levels of gastric MDA content by 36.9%, 34.4%, 38.5%, 32.9%, and 34.5%, respectively in relative to MDA levels in the INDO ulcer control group, also EA and COQ10 and COQ10+RA pre-treatment administration had caused significant different effect compared to MDA levels in normal control group. (Figure 3).

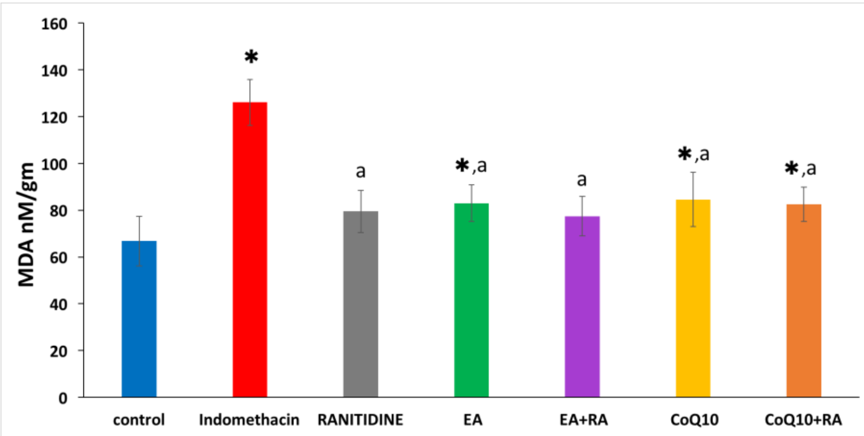

Figure 3. Effects of pre-treatment of EA and CoQ10 each alone and in combination with RA on gastric MDA content. Data are expressed as mean (± SD) (n= 8 rats). Values are significantly different from normal control* or INDO[a] only (ulcer) (p-value ≤ 0.05).

3.4. Effect on gastric NO

INDO administration significantly (p-value ≤ 0.05) dropped the level of NO content in stomach tissue by 46.2%, compared to that in gastric tissues of the normal control rats Group. (Table 2).

Table 2: Effects of pre-treatment of EA and CoQ10 each alone and in combination with RA on gastric NO, SOD, and TNF-α.

GROUP/ PARAMETER	NO µmol/ gm	SOD U/gm	TNF-α pg/gm
CONTROL	21.51±2.64	28.74±2.45	177.24±45.32
INDO	11.57±1.25*	17.51±1.47*	583.91±62.34*
RA	21.93±3.35[a]	26.28±2.49[a]	157.05±56.20[a]
EA	16.74±3.56*[,a,b]	24.89±1.94*[,a]	198.91±58.17[a]
EA+RA	23.38±3.45[a,c]	26.81±1.88[a]	159.53±24.39[a]
CoQ10	19.69±1.54[a]	25.29±1.98*[,a]	160.26±52.32[a]
CoQ10+RA	20.83±2.92[a]	25.97±1.49[a]	153.75±26.07[a]

*****p ≤ 0.05, significant with control (I) group.
[a]p ≤ 0.05, significant with INDO-induced ulcer group (II).
[b]p ≤ 0.05, significant wirh RA + INDO group (III).
[c]p ≤ 0.05, significant with EA group (IV).

Pre-treatments with RA, EA, EA+RA, COQ10, and COQ10+RA each alone produced significantly increased gastric tissue content levels of NO content by 48.4%, 44.6%, 50.5%, 41.2%, and 44.4% respectively, relative to the level in the INDO only ulcer control group, also EA pre-treatment administration results in a significantly different effect on NO level relative to NO levels in the normal control group, RA+INDO group and EA+RA+INDO group (Figure 4).

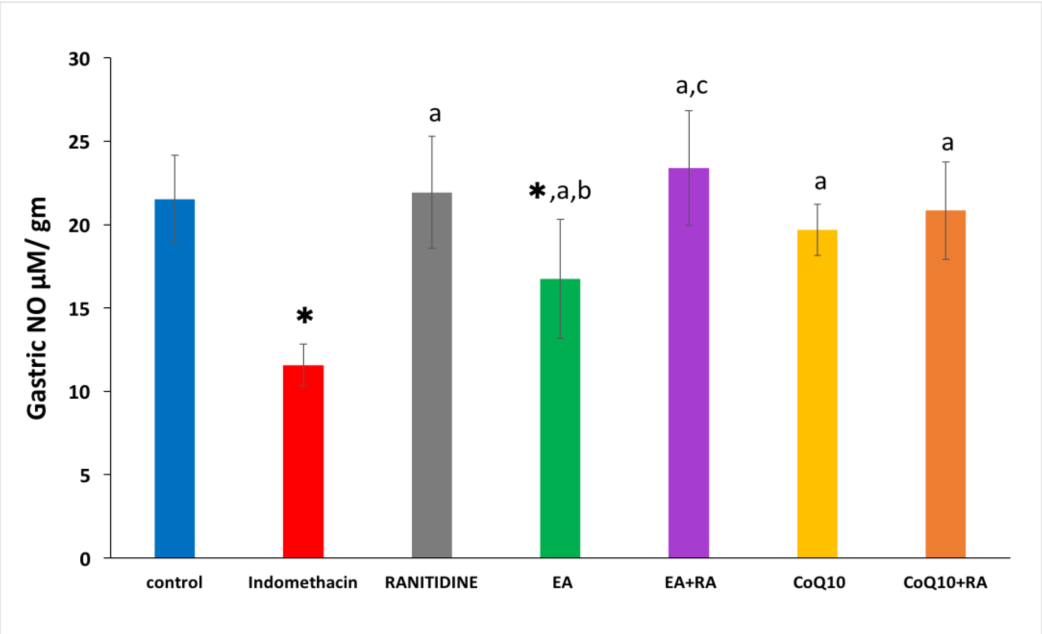

Figure 4. Effects of pre-treatment of EA and CoQ10 each alone and in combination with RA on gastric NO content. Data are expressed as mean (± SD) (n= 8 rats). Values are significantly different from normal control* or INDO[a] only (p-value ≤ 0.05).

3.5. Effect on gastric SOD

INDO administration to ulcer control rats significantly lowered (p-value ≤ 0.05) the SOD activity in gastric tissue, specifically decreased by 39.0% compared to SOD activity in gastric tissues of normal control rats. (table 2).

Pre-treatments with RA, EA, EA+RA, COQ10, and COQ10+RA separately produced significantly increased activity levels of SOD in gastric tissue by 33.3%, 29.6%, 34.4%, 30.2%, and 32.0% respectively, relative to its activity level in the INDO only ulcer group. Also, EA and COQ10 pre-treatment administration to rats had caused a significantly different effect on SOD activity compared to SOD activity in the normal control group (Figure 5).

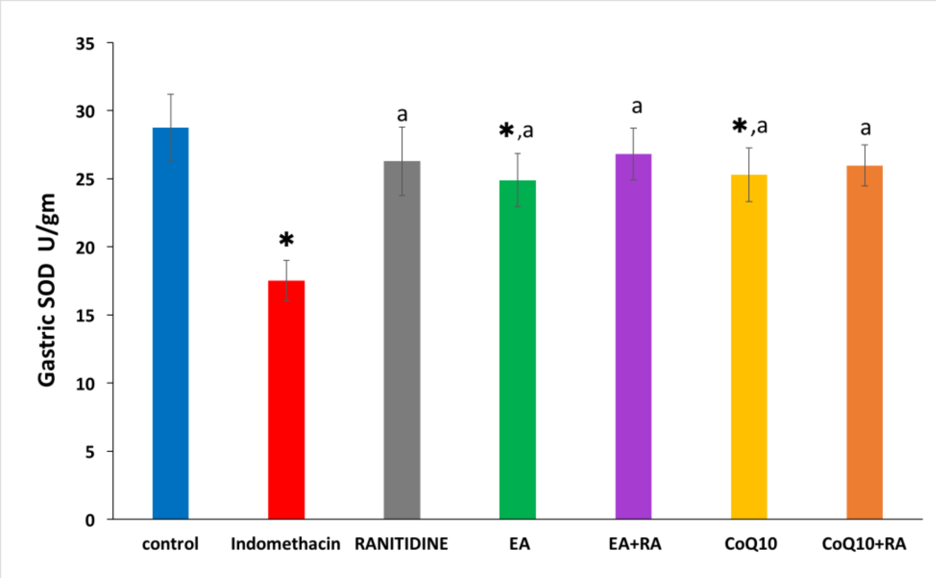

Figure 5. Effects of pre-treatment of EA and CoQ10 each alone and in combination with RA on gastric SOD activity. Data are expressed as mean (± SD) (n= 8 rats). Values are significantly different from normal control* or INDO[a] only (p-value ≤ 0.05).

3.6. Effect on gastric TNF-α

INDO alone administration significantly increased gastric TNF-α content; levels were increased by 229.2% compared to the TNF-α level in tissue samples of the normal control group (Table 2).

Pre-treatment with RA, EA, EA+RA, COQ10, and COQ10+RA separately significantly decreased gastric TNF-α levels by 271.8%, 193.5%, 266.0%, 264.3%, and 297.7%, respectively, relative to rats in ulcer group that received INDO alone. Moreover, no significant differences in TNF-α content level among rats that pre-treated with EA, EA+RA, COQ10, and COQ10+RA and that pre-treated with RA. (Figure 6).

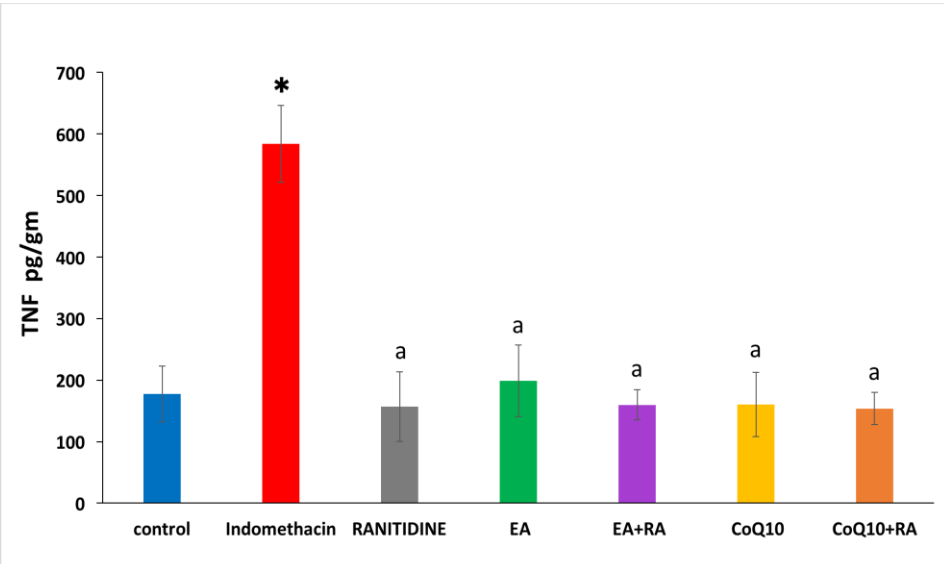

Figure 6. Effects of pre-treatment of EA and CoQ10 each alone and in combination with RA on gastric TNF-α content. Data are expressed as mean (± SD) (n= 8 rats). Values are significantly different from normal control* or INDO[a] only (p-value ≤ 0.05).

3.7. Effect on tissue histopathology

Normal control group I sections displayed normal (intact) gastric mucosal and submucosal layers architectures (Figure 7A). INDO ulcerated rats of group II displayed tissues with erosions, decreased mucosal thickness, gastric mucosal disruption, necrosis, and deep ulcerations. (Figure 7 B1, B2).

Pre-treatment with RA, EA, EA+RA, COQ10, and COQ10+RA separately noticeably reduced INDO-induction of stomach ulcerations and led to nearly normal gastric mucosal, submucosal architectures, and kept the gastric mucosal tissue within the normal limits.

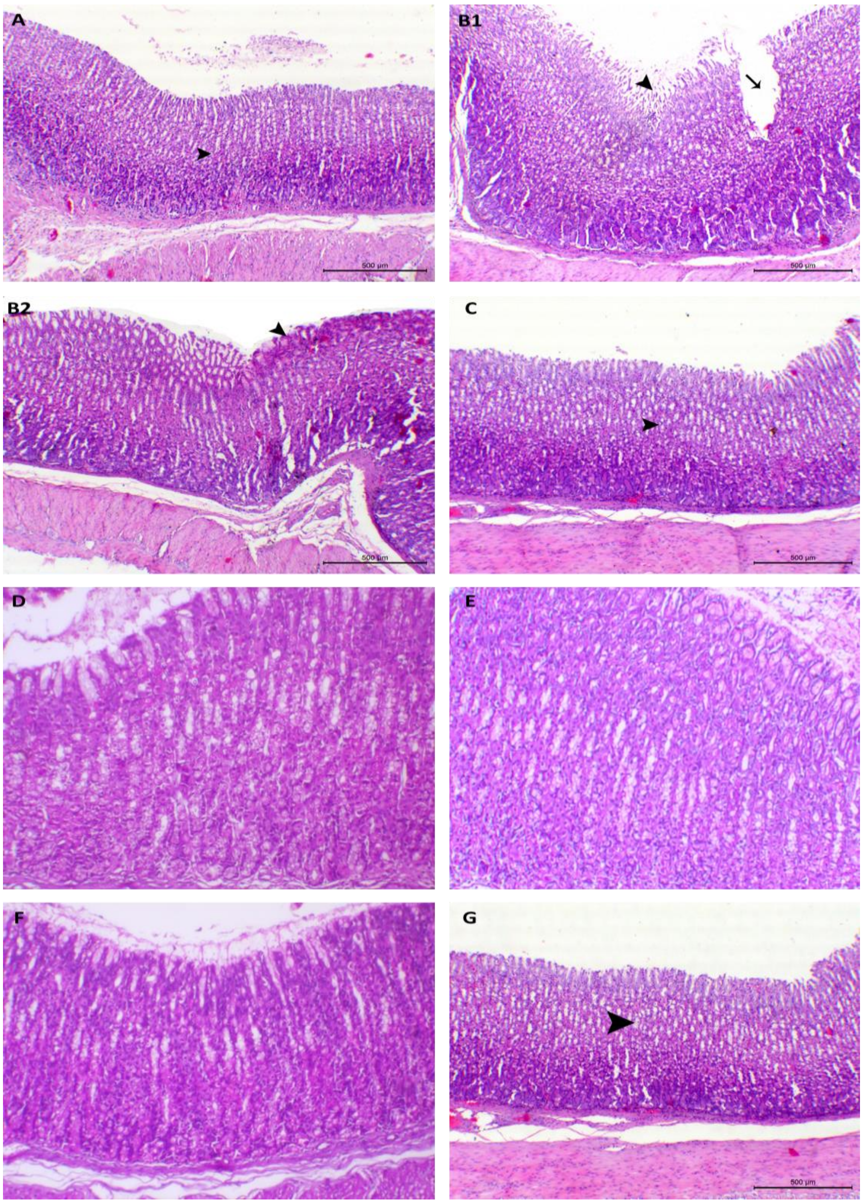

Figure 7: Histological evaluations of stomach tissues stained with H&E (X 100). Effects of pre-treatment of EA and CoQ10 each alone and in combination with RA on the histopathological changes in gastric tissue of INDO ulcerated rats. Section (A) of normal group indicates normal mucosal thickness and intact mucosa. Both sections (B1) and (B2) of ulcer control group indicates a decrease in thickness of mucosal layer, loss of epithelial layer, loss of normal glandular architectures, erosions and deep ulceration. Sections (C), (D), (E), (F) and (G) gastric tissues section of rat indicates gastric mucosa within normal limits.

3.8. Effect on apoptosis

Immunohistochemical staining for detection of caspase-3 in gastric tissues showed that INDO alone administration to rats in group II significantly enhanced mucosal up-regulation of caspase-3 activities compared to normal control group II. In the normal control group, the expression of caspase-3 was markedly low (Table 3).

Pre-treatment with RA, EA, EA+RA, COQ10, and COQ10+RA each alone reduced the increment in caspase-3 up-regulation produced by INDO and resulted in significant mild expression of caspase-3 relative to the ulcer group (Figure 8,9).

Table 3: Effects of pre-treatment of EA and CoQ10 each alone and in combination with RA on caspase-3.

Group/ Parameter	Area percentage of immune-positive cell
CONTROL	3.11 % ± 1.479
INDO	23.02 % ± 2.497*
RA	3.80 % ± 1.944*[,a]
EA	4.73 % ± 1.886*[,a,b]
EA+RA	4.01 % ± 1.984*[,a,c]
CoQ10	4.52 % ± 1.497*[,b,d]
CoQ10+RA	3.55 % ± 2.454*[,c,e]

Data expressed as means ± SD

***** $p \leq 0.05$, significant vs normal control (I) group.

[a]p ≤ 0.05, significant vs INDO-induced ulcer group (II).
[b]p ≤ 0.05, significant vs RA + INDO group (III).
[c]p ≤ 0.05, significant vs EA group (IV).
[d]p ≤ 0.05, significant vs EA+RA group (V).
[e]p ≤ 0.05, significant vs COQ10 group (VI).

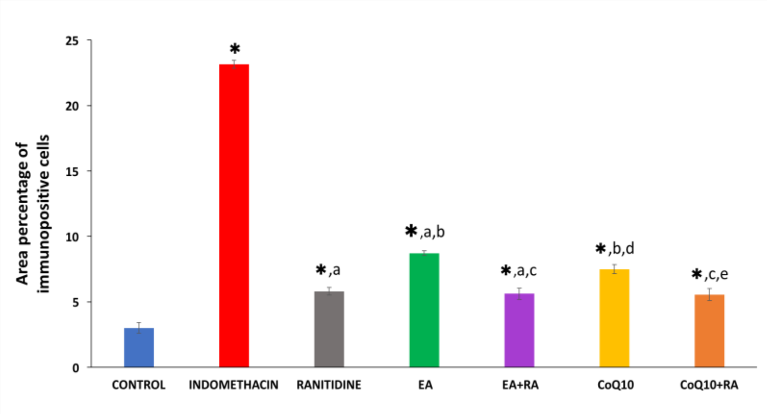

Figure 8: Effects of pre-treatment of EA and CoQ10 each alone and in combination with RA on apoptotic changes. Data are expressed as mean (± SD) (n= 8 rats). Values are significantly different from normal control* or INDO[a] only, Data presented as means ± SD (p-value ≤ 0.05).

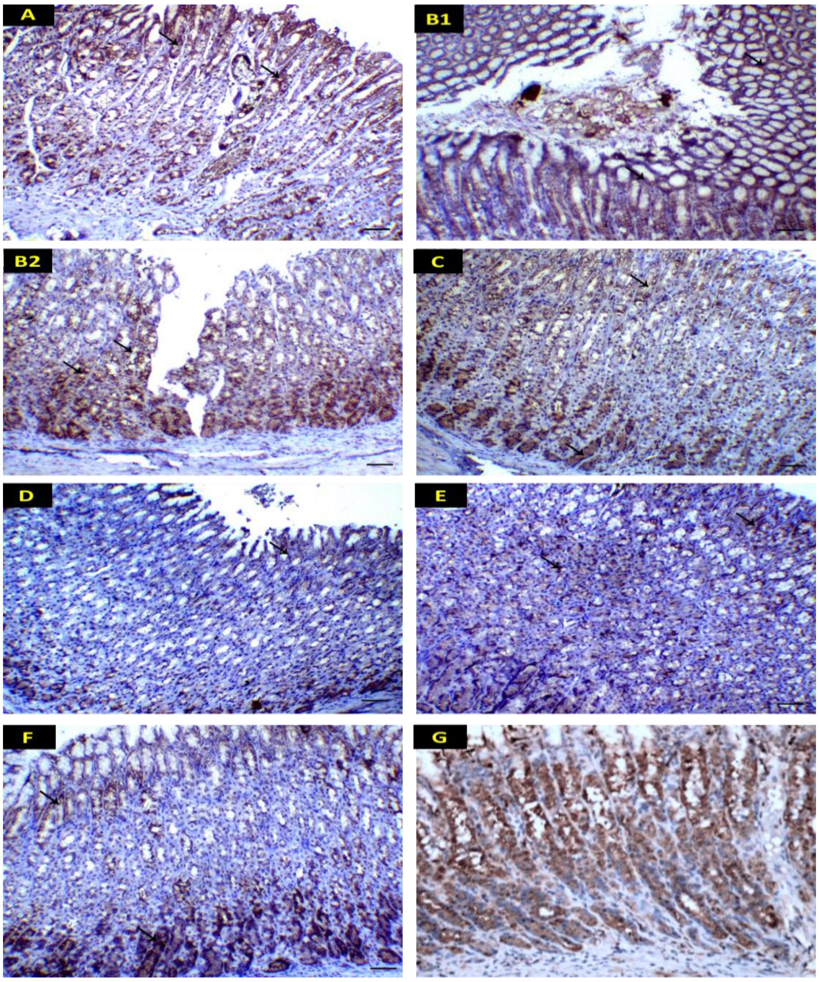

Figure 9: Immuno-histochemical staining of gastric sections of: (A) group I normal animal exhibit mild expression of caspase-3 inside the gastric mucosa (arrows); (B1) group II INDO treated animal showing gastric ulcer with marked severe expression of caspase-3 inside the gastric glands lining epithelium (arrows); (B2) group II INDO treated animal showing deep ulceration with positive caspase-3 expression mostly around ulcerated portion (arrows); (C) group III RA pre-treated animal exhibit decline in of caspase-3 positive expression within the gastric mucosa compared to group II (arrows); (D) ; (E) group IV and group V of EA or COQ10 pre-treated animals respectively showing mild expression of

caspase-3 inside the gastric glands lining epithelium, which is markedly decreased compared to group II (arrows); (F) group VI EA+RA-treated animal showing marked decrease in the positive expression of caspase-3 within the gastric mucosa (arrows); (G) group VII COQ10+RA treated animal showing marked decrease the positive expression of caspase-3 within the gastric mucosa (arrows), caspase-3 IHC, bar= 50 µm**.**

4. DISCUSSION

The imbalance between gastro-protective and destructive agents in the gastric mucosa result in gastric damage and deep ulceration.[42]

Endogenous gastro-protective factors embrace NO, and PGs are an important regulator of mucosal blood flow, bicarbonate, and mucus production and secretion.[43]

Since gastric pH, NO, PGs, TNF-α, Caspase-3, and oxidative stress have been connected with gastric ulcers[44], one can assume that the use of anti-inflammatory, antioxidant drugs with pH buffering effects should be useful for retaining the natural physiological balance in the gastric mucosa and preventing the growth and expansion of ulcer.

This study aimed to investigate the potential protective effect of Ellagic acid and Coenzyme Q10, each alone or in combination with ranitidine, against INDO-induced gastric ulcers and explore the possible mechanisms of these effects.

In the current work, stomach ulcers were induced by indomethacin (INDO) and were confirmed with histopathological evaluation. The gastric tissues of INDO-administered rats showed evidence of mucosal damage, deteriorated epithelial layers, deformation of the mucosal glands and mucosa, diminished thickness of the mucosal layer, and inflammatory cell infiltrate. In addition, INDO-induced ulcers have been correlated with significant increases in gastric acidity. Excess gastric acid secretion plays a crucial role in the induction of stomach ulcers. It is implicated in the etiology of ulcers as it diminishes the process of restoration and ulcer healing.[45]

INDO administration to rats in the present study exhibited a significant drop in gastric pH and NO content. These findings supported that of earlier researchers[46], who recognized that stomach ulcers caused by INDO have been concomitant with marked increases in stomach acidity and a significant drop in gastric levels of NO. This effect on NO level could be elucidated by INDO's ability to increase endothelin-1expression, which led to depression in the release of endothelial NO, resulting in loss of mucosa integrity.[47]

The current results also exhibited that the pre-treatment of EA and CoQ10, each alone and in combination with RA before INDO, normalized gastric acidity; this consequence could be elucidated by the ability of these pre-treatments to raise NO and PGE2 levels; this is because PGE2 and NO are known to increase mucus production and secretion and regulate the acidity of the stomach by reducing gastric acid secretion[48], where some reports clarified that the NO and COX enzymes interactions were mutually positive, which working together in protection of the gastric tissue from injury.[49,50]

The detected effect of either EA or CoQ10 was approximately close to that exerted by the H2 blocker; ranitidine, which increased the pH value without any significant difference compared to either EA or CoQ10 to one greater than normal, and the greatest increment in the pH was exerted by the combination of RA with either EA or CoQ10.

Even though NO is a form of ROS, any oxidative stress connected with its existence must not be implicated in gastric tissue injury.[51]

ROS could cause injuries to the gastric mucosal tissue by causing damage to membranes and cellular molecules such as DNA, proteins, and lipids.[52] Moreover, the increase in ROS levels leads to the uncoupling of eNOS and diminishes NO synthesis.[53] Thereby, drugs that possess antioxidant and anti-inflammatory properties, which decrease the formation of ROS and increase levels of NO, could prevent gastric tissues from damage and be beneficial against the emergence and expansion of ulcers.

The results here also revealed that gastric NO content was increased significantly in the EA and CoQ10, each alone group, or in the combination of EA or CoQ10 with RA compared to the INDO-only group.

Furthermore, RA significantly raised gastric NO content relative to the INDO group. This finding was by earlier studies[54] that RA raised NO level values and contributed to a gastro-protective effect against ulcers induced with INDO in the stomachs of rats. Each EA or CoQ10 alone or combined with RA pre-treatments here caused similar results to RA and assisted rats in preserving stomach NO levels close to normal rat values.

Oxidative stress is implied in the induction of ulcers in stomach tissue and is counted among the mechanisms implicated in ulcer induction with INDO.[55] The current results propped this assumption as MDA content significantly raised. In contrast, GSH content and SOD were remarkably decreased in the gastric tissues of rats treated only with INDO relative to the values of normal rats.

Ellagic acid and Coenzyme Q10 have an antioxidant effect, maybe by inhibiting NADPH oxidase-induced formation of free radicals, direct scavenging of ROS[56,57], and hem oxygenase-1 (HO-1) enzyme up-regulation, which increase SOD and GSH levels.[58,59]

In the present study, EA or CoQ10, each alone pre-treatment or in combination with RA prior to INDO, caused significant increases in stomach SOD and GSH contents and marked decreases in MDA contents of stomach tissue relative to that found in INDO-only treated rats.

Ranitidine pre-treatment, here, the effect was the same as that of EA and CoQ10 separately on GSH, SOD, and MDA, while the combination of RA with EA or CoQ10 had a higher effect on GSH, SOD, and MDA levels. RA restored all of them to near-normal levels, whereas RA+EA and CoQ10+RA each corrected and restored the MDA and GSH contents to better levels than normal. This effect was consistent with earlier reports[60], which suggest that ranitidine can replenish GSH levels in restraint cold stress-induced ulcer models.

Antioxidant Activity of RA could be elucidated by its strong scavenging effect of hypochlorous acid (HOCl) and monochloramine, which emerge from inflammatory cells like neutrophils, scavenge free radicals, restore endogenous antioxidant levels, chelation of Fe^{+2} and hydroxyl radicals generated on $Fe^{+2}.H_2O_2$ reaction mixture[61] and enhancing expression of hemoxygenase-1 (HO-1) enzyme.[62]

In ulcerated tissues, INDO was shown to up-regulate the production of TNF-α.[63] TNF-α involvement in INDO-induced gastric ulcer emergence and development was confirmed in this study, and that coincided with.[64]

TNF-α plays a considerable role in gastric ulcers induced by INDO; this mediator causes inflammation and tissue destruction by activation of up-regulation of adherent molecules, thus leading to an infiltration of neutrophils that cause damage by increasing the release of ROS, which damages gastric tissue.[65] On the other side, the up-regulatory influence of INDO on TNF-α may be responsible for lowering the NO level in the mucosal tissue of the stomach; This was to previously reported results[66], which recorded that TNF-α is a strong inhibitor of constitutive NO, which mediated a protective effect on the stomach. It has been provided previously that ranitidine decreased TNF-α levels and was protective against INDO-induced gastric ulcers.[67] EA and CoQ10 could inhibit TNF-α gene expression.[68,69] Lastly, PGE2 attenuates the effects of TNF-α and has been reported as a powerful inhibitor of TNF-α.[70] The presented work revealed that pre-treatments of rats with RA, EA, CoQ10, RA+EA, and CoQ10+RA alone significantly decreased TNF-α levels contents in the stomach compared to that in rats administrated INDO. The decline in TNF-α levels produced by these pre-treatments remains slightly higher than the TNF-α contents of the normal rat group. Our findings establish that pre-treatment with EA and CoQ10 alone could act as anti-inflammatory agents on gastric ulcers. Also, we indicated that the ulcer protection effect of EA and CoQ10 is mediated by decreasing TNF-α, an important cytokine for the gastric injury process.

Enhancement of caspase-3 activity and gastric cell apoptosis are major pathological incidents characterizing NSAID-induced mucosal injury.[71]

Earlier studies demonstrated that, antioxidant, and anti-inflammatory drugs decreased the elevation of gastric caspase-3 activity in rats with stomach ulcers induced by INDO.[72]

TNF-α was remarkably increased in ulcerated mucosa of rat stomach in the attendant study, and ROS, which is no doubt overproduced by the infiltrating inflammatory cells, has been presumed to perform a critical role in INDO-induced apoptosis of gastric mucosa[73],

EA and CoQ10 are thought to hold anti-apoptotic activity, likely by inhibiting TNF-α up-regulation of expression, direct scavenging of ROS, and elevating of NO content levels[74,75],

Our findings that enhanced apoptosis was coupled with elevated TNF-α levels establish other studies suggesting that TNF-α activation mediates apoptosis in gastric epithelial cells.[76]

Therefore, the observed suppressive effect of EA and CoQ10 on TNF-α signaling might further contribute to their anti-apoptotic potentiality.

In the present study, pre-treatment with either EA or CoQ10 alone or combination with RA before INDO significantly lowered caspase-3 relative to those found in INDO-only rats. Ranitidine pre-treatment has the same effect as that of EA and CoQ10 separately on caspase-3, while the combination of RA with either EA or CoQ10 had a higher effect on caspase-3. This effect was in line with that cited in earlier reports.[77]

This study substantiates a potent gastro-protection of EA and CoQ10 against INDO-induced gastric mucosal ulcers with comparable efficacy to ranitidine.

5. REFERENCE

1. Motawi, Tarek K., Hanan M. Abd Elgawad, and Nancy N. Shahin. "Modulation of indomethacin-induced gastric injury by spermine

and taurine in rats." *Journal of biochemical and molecular toxicology*, 2007; 21.5: 280-288.
2. Boligon, Aline Augusti, Michel Mansur Machado, and Margareth Linde Athayde. "Technical evaluation of antioxidant activity." *Med chem.*, 2014; 4.7: 517-522.
3. Borrelli, Francesca, and Angelo A. Izzo. "The plant kingdom as a source of anti-ulcer remedies." *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, 2000; 14.8: 581-591.
4. Takeuchi, Koji. "Pathogenesis of NSAID-induced gastric damage: importance of cyclooxygenase inhibition and gastric hypermotility." *World journal of gastroenterology: WJG*, 2012; 18.18: 2147.
5. Farzaei, Mohammad Hosein, et al. "An evidence-based review on medicinal plants used for the treatment of peptic ulcer in traditional Iranian medicine." *Int J Pharmacol*, 2013; 9.2: 108-124.
6. Wallace, John L. "Prostaglandins, NSAIDs, and gastric mucosal protection: why doesn't the stomach digest itself?" *Physiological reviews*, 2008; 88.4: 1547-1565.
7. Koizumi, Shigeto, et al. "Attenuation of gastric mucosal inflammation induced by indomethacin through activation of the A 2A adenosine receptor in rats." *Journal of gastroenterology*, 2009; 44.5: 419-425.
8. Neu, Bruno, et al. "TNF-α induces apoptosis of parietal cells." *Biochemical pharmacology*, 2003; 65.10: 1755-1760.
9. Naito, Yuji, and Toshikazu Yoshikawa. "Oxidative stress involvement and gene expression in indomethacin-induced gastropathy." *Redox Report*, 2006; 11.6: 243-253.
10. Wallace, JOHN L., CATHERINE M. Keenan, and D. NEIL Granger. "Gastric ulceration induced by nonsteroidal anti-inflammatory drugs is a neutrophil-dependent process." *American Journal of Physiology-Gastrointestinal and Liver Physiology*, 1990; 259.3: G462-G467.
11. Suleyman, Halis, et al. "Different mechanisms in formation and prevention of indomethacin-induced gastric ulcers." *Inflammation*, 2010; 33.4: 224-234.
12. Araujo, Deborah AO, et al. "Gastroprotective effects of essential oil from Protium heptaphyllum on experimental gastric ulcer models in rats." *Revista Brasileira de Farmacognosia*, 2011; 21.4: 721-729.
13. Mejia, Alex, and Walter K. Kraft. "Acid peptic diseases: pharmacological approach to treatment." *Expert review of clinical pharmacology*, 2009; 2.3: 295-314.
14. Vattem, D. A., and K. Shetty. "Biological functionality of ellagic acid: a review." *Journal of food biochemistry*, 2005; 29.3: 234-266.
15. Majid, Sabhiya, et al. "Influence of ellagic acid on antioxidant defense system and lipid peroxidation in mice." *Biochemical pharmacology*, 1991; 42.7: 1441-1445.
16. Cozzi, R., et al. "Taurine and ellagic acid: Two differently-acting natural antioxidants." *Environmental and molecular mutagenesis*, 1995; 26.3: 248-254.
17. H Farzaei, Mohammad, Roja Rahimi, and Mohammad Abdollahi. "The role of dietary polyphenols in the management of inflammatory bowel disease." *Current pharmaceutical biotechnology*, 2015; 16.3: 196-210.
18. H Farzaei, Mohammad, Roja Rahimi, and Mohammad Abdollahi. "The role of dietary polyphenols in the management of inflammatory bowel disease." *Current pharmaceutical biotechnology*, 2015; 16.3: 196-210.
19. Chatterjee, Ananya, Sirshendu Chatterjee, and S. K. Bandyopadhyay. "H. pylori-induced gastric ulcer: pathophysiology and herbal remedy." *Int J Biol Med Res.*, 2012; 3.1: 1461-5.
20. Ogawa, Yoshihiro, et al. "Protection against dextran sulfate sodium-induced colitis by microspheres of ellagic acid in rats." *Life sciences*, 2002; 71.7: 827-839.
21. Banerjee, Debashish, et al. "Gastroprotective properties of Myristica malabarica against indometacin-induced stomach ulceration: a mechanistic exploration." *Journal of Pharmacy and Pharmacology*, 2007; 59.11: 1555-1565.
22. Kamei, Junzo, et al. "Therapeutic potential of PKC inhibitors in painful diabetic neuropathy." *Expert opinion on investigational drugs*, 2001; 10.9: 1653-1664.
23. Crane, Frederick L. "Biochemical functions of coenzyme Q10." *Journal of the American College of Nutrition*, 2001; 20.6: 591-598.
24. Schmelzer, Constance, et al. "Functions of coenzyme Q10 in inflammation and gene expression." *Biofactors*, 2008; 32.1-4: 179-183.
25. Rauscher, Frederick M., Ruth A. Sanders, and John B. Watkins III. "Effects of coenzyme Q10 treatment on antioxidant pathways in normal and streptozotocin-induced diabetic rats." *Journal of biochemical and molecular toxicology*, 2001; 15.1: 41-46.
26. Beal, M. Flint, et al. "Coenzyme Q10 and nicotinamide block striatal lesions produced by the mitochondrial toxin malonate." *Annals of Neurology: Official Journal of the American Neurological Association and the Child Neurology Society*, 1994; 36.6: 882-888.
27. Watts, G. F., et al. "Coenzyme Q10 improves endothelial dysfunction of the brachial artery in Type II diabetes mellitus." *Diabetologia*, 2002; 45.3: 420-426.
28. Papucci, Laura, et al. "Coenzyme q10 prevents apoptosis by inhibiting mitochondrial depolarization independently of its free radical scavenging property." *Journal of Biological Chemistry*, 2003; 278.30: 28220-28228.
29. Araujo, Deborah AO, et al. "Gastroprotective effects of essential oil from Protium heptaphyllum on

experimental gastric ulcer models in rats." *Revista Brasileira de Farmacognosia*,. 2011; 21.4: 721-729.
30. Süleyman, Halis, Fatih Akçay, and Konca Altinkaynak. "The effect of nimesulide on the indomethacin-and ethanol-induced gastric ulcer in rats." *Pharmacological research*, 2002; 45.2: 155-158.
31. Corbett, Stephanie, et al. "Evaluation of the anti-inflammatory effects of ellagic acid." *Journal of PeriAnesthesia Nursing*, 2010; 25.4: 214-220.
32. Beal, M. Flint, et al. "Coenzyme Q10 and nicotinamide block striatal lesions produced by the mitochondrial toxin malonate." *Annals of Neurology: Official Journal of the American Neurological Association and the Child Neurology Society*, 1994; 36.6: 882-888.
33. Ismail, Iza Farhana, et al. "Gastroprotective activity of Polygonum chinense aqueous leaf extract on ethanol-induced hemorrhagic mucosal lesions in rats." *Evidence-Based Complementary and Alternative Medicine*, 2012; 2012.
34. Ramesh, A., N. Alekhya, and I. Iohitha. "Antiulcer activity of Eugenia jambolana leaves against ethanol induced gastric ulcer in albino rats." *Int J Pharm Res Dev*, 2011; 3: 106-112.
35. Sakat, S. S., Preeti Tupe, and Archana Juvekar. "Gastroprotective effect of Oxalis corniculata (whole plant) on experimentally induced gastric ulceration in Wistar rats." *Indian journal of pharmaceutical sciences*, 2012; 74.1: 48.
36. Muniappan, M., and T. Sundararaj. "Antiinflammatory and antiulcer activities of Bambusa arundinacea." *Journal of ethnopharmacology*, 2003; 88.2-3: 161-167.
37. Ellman, George L. "Tissue sulfhydryl groups." *Archives of biochemistry and biophysics*, 1959; 82.1: 70-77.
38. Ohkawa, Hiroshi, Nobuko Ohishi, and Kunio Yagi. "Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction." *Analytical biochemistry*, 1979; 95.2: 351-358.
39. Montgomery, H. A. C. D. J., and Joan F. Dymock. "Determination of nitrite in water." *Analyst*, 1961; 86.102: 414.
40. Nishikimi, Morimitsu, N. Appaji Rao, and Kunio Yagi. "The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen." *Biochemical and biophysical research communications*, 1972; 46.2: 849-854.
41. Saber, Sameh, et al. "Olmesartan ameliorates chemically-induced ulcerative colitis in rats via modulating NFκB and Nrf-2/HO-1 signaling crosstalk." *Toxicology and applied pharmacology*, 2019; 364: 120-132.
42. Raji, Yinusa, and Ganiyat Kehinde Oloyede. "Antiulcerogenic effects and possible mechanism of action of Quassia amara (L. Simaroubaceae) extract and its bioactive principles in rats." African Journal of Traditional, Complementary *and Alternative Medicines*, 2012; 9.1: 112-119.
43. Suleyman, Halis, et al. "Different mechanisms in formation and prevention of indomethacin-induced gastric ulcers." Inflammation, 2010; 33.4: 224-234.
44. Giles, Thomas D., et al. "Impaired vasodilation in the pathogenesis of hypertension: focus on nitric oxide, endothelial-derived hyperpolarizing factors, and prostaglandins." The Journal of Clinical Hypertension, 2012; 14.4: 198-205.
45. Musumba C, Pritchard DM, Pirmohamed M. Cellular and molecular mechanisms of NSAID-induced peptic ulcers. Aliment Pharmacol Ther., 2009; 30: 517–531.
46. Abbas, Amr M., and Hussein F. Sakr. "Effect of selenium and grape seed extract on indomethacin-induced gastric ulcers in rats." Journal of physiology and biochemistry, 2013; 69.3: 527-537.
47. Abdel-Raheem, Ihab T. "Gastroprotective effect of rutin against indomethacin-induced ulcers in rats." Basic & clinical pharmacology & toxicology, 2010; 107.3: 742-750.
48. Suleyman, Halis, et al. "Different mechanisms in formation and prevention of indomethacin-induced gastric ulcers." Inflammation, 2010; 33.4: 224-234.
49. Mollace, Vincenzo, et al. "Modulation of prostaglandin biosynthesis by nitric oxide and nitric oxide donors." Pharmacological reviews, 2005; 57.2: 217-252.
50. Usman, Dabo, Oluwole Frank Sunday, and Adedeji Temitope Gabriel. "Effects of L-arginine and L-citrulline on indomethacin-induced gastric ulceration and gastric pH in male albino rats." Europ J of Medici Plan, 2014; 4.6: 623-640.
51. Wynn, Thomas A. "Cellular and molecular mechanisms of fibrosis." The Journal of Pathology: A Journal of the Pathological Society of Great Britain and Ireland, 2008; 214.2: 199-210.
52. Badr, Gehan Moustafa, and Jawaher Abdulaziz AL-Mulhim. "The protective effect of aged garlic extract on nonsteroidal anti-inflammatory drug-induced gastric inflammations in male albino rats." Evidence-Based Complementary and Alternative Medicine, 2014; 2014.
53. Kato, S., et al. "Role of endothelial nitric oxide synthase in aggravation of indomethacin-induced gastric damage in adjuvant arthritic rats." J Physiol Pharmacol, 2009; 60.4: 147-155.
54. Freitas, Cristina Setim, et al. "Involvement of nitric oxide in the gastroprotective effects of an aqueous extract of Pfaffia glomerata (Spreng) Pedersen, Amaranthaceae, in rats." Life Sciences, 2004; 74.9: 1167-1179.
55. Adhikary, Biplab, et al. "Black tea and theaflavins assist healing of indomethacin-induced gastric ulceration in mice by antioxidative action." Evidence-based complementary and alternative medicine, 2011; 2011.
56. Lee, Wen-Jane, et al. "Ellagic acid inhibits oxidized LDL-mediated LOX-1 expression, ROS generation, and inflammation in human endothelial

cells." Journal of vascular surgery, 2010; 52.5: 1290-1300.
57. Papucci, Laura, et al. "Coenzyme q10 prevents apoptosis by inhibiting mitochondrial depolarization independently of its free radical scavenging property." Journal of Biological Chemistry, 2003; 278.30: 28220-28228.
58. Aggarwal, Bharat, et al. "Prevention and treatment of colorectal cancer by natural agents from mother nature." Current colorectal cancer reports, 2013; 9.1: 37-56.
59. Choi, Hoo-Kyun, et al. "Inhibition of liver fibrosis by solubilized coenzyme Q10: role of Nrf2 activation in inhibiting transforming growth factor-β1 expression." Toxicology and applied pharmacology, 2009; 240.3: 377-384.
60. Bandyopadhyay, Debashis, et al. "Melatonin protects against gastric ulceration and increases the efficacy of ranitidine and omeprazole in reducing gastric damage." Journal of pineal research, 2002; 33.1: 1-7.
61. van Zyl, Johann M., André Kriegler, and Ben J. van Der Walt. "Anti-oxidant properties of H2-receptor antagonists: Effects on myeloperoxidase-catalysed reactions and hydroxyl radical generation in a ferrous-hydrogen peroxide system." Biochemical pharmacology, 1993; 45.12: 2389-2397.
62. Schulz-Geske, Stephanie, et al. "Molecular mechanism and functional consequences of lansoprazole-mediated heme oxygenase-1 induction." World Journal of Gastroenterology: WJG, 2009; 15.35: 4392.
63. Abdallah IZ, Khattab HA, Heeba GH. Gastroprotective effect of Cordia myxa L. fruit extract against indomethacin-induced gastric ulceration in rats. Life Sci J., 2011; 8: 433–445.
64. Appleyard, C. B., et al. "Tumor necrosis factor mediation of NSAID-induced gastric damage: role of leukocyte adherence." American Journal of Physiology-Gastrointestinal and Liver Physiology, 1996; 270.1: G42-G48
65. Musumba, C., D. M. Pritchard, and M. Pirmohamed. "cellular and molecular mechanisms of NSAID-induced peptic ulcers." Alimentary pharmacology & therapeutics, 2009; 30.6: 517-531.
66. Bauer, H., et al. "Nitric oxide inhibits the secretion of T-helper 1-and T-helper 2-associated cytokines in activated human T cells ‘pa." Immunology, 1997; 90.2: 205-211.
67. Tukov, Francis F., et al. "The role of tumor necrosis factor alpha in lipopolysaccharide/ranitidine-induced inflammatory liver injury." Toxicological sciences, 2007; 100.1: 267-280.
68. Umesalma, Syed, and Ganapasam Sudhandiran. "Differential inhibitory effects of the polyphenol ellagic acid on inflammatory mediators NF-κB, iNOS, COX-2, TNF-α, and IL-6 in 1, 2-dimethylhydrazine-induced rat colon carcinogenesis." Basic & clinical pharmacology & toxicology, 2010; 107.2: 650-655.
69. Abdollahzad, Hadi, et al. "Effects of coenzyme Q10 supplementation on inflammatory cytokines (TNF-α, IL-6) and oxidative stress in rheumatoid arthritis patients: a randomized controlled trial." Archives of medical research, 2015; 46.7: 527-533.
70. Abood, Walaa Najm, Mahmood A. Abdulla, and Salmah Ismail. "Involvement of inflammatory mediators in the gastroprotective action of Phaleria macrocarpa against ethanol-induced gastric ulcer." World Applied Sciences Journal, 2014; 30: 344-350.
71. Slomiany, B. L., J. Piotrowski, and Amalia Slomiany. "Role of caspase-3 and nitric oxide synthase-2 in gastric mucosal injury induced by indomethacin: effect of sucralfate." *Journal of physiology and pharmacology: an official journal of the Polish Physiological Society*, 1999; 50.1: 3-16.
72. Shalkami, A. S., M. I. A. Hassan, and A. G. Bakr. "Anti-inflammatory, antioxidant and anti-apoptotic activity of diosmin in acetic acid-induced ulcerative colitis." *Human & experimental toxicology*, 2018; 37.1: 78-86.
73. Fukumoto, Kohei, et al. "Role of tumor necrosis factor-α in the pathogenesis of indomethacin-induced small intestinal injury in mice." *International journal of molecular medicine*, 2011; 27.3: 353-359.
74. Jing, Li, et al. "Coenzyme Q10 protects astrocytes from ROS-induced damage through inhibition of mitochondria-mediated cell death pathway." *International journal of biological sciences*, 2015; 11.1: 59.
75. Tsai, Kun-Ling, et al. "A novel mechanism of coenzyme Q10 protects against human endothelial cells from oxidative stress-induced injury by modulating NO-related pathways." *The Journal of nutritional biochemistry*, 2012; 23.5: 458-468.
76. Szabo, I., and A. S. Tarnawski. "Apoptosis in the gastric mucosa: molecular mechanisms, basic and clinical implications." *Journal of Physiology and Pharmacology*, 2000; 51.1.
77. Park, Hae Jeong, et al. "Protective effect of histamine H2 receptor antagonist ranitidine against rotenone-induced apoptosis." *Neurotoxicology*, 2009; 30.6: 1114-1119.