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ORIGINAL ARTICLE

Emblica officinalis Fruit Pulp Extract Protects against Naproxen-Induced Gastric Ulcer in Rats: Anti-inflammatory and Antioxidant Machinery

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ABSTRACT

Background: The use of non-steroidal anti-inflammatory drugs (NSAIDs) is one of the leading causes of peptic ulcers. There is a rising interest and demand for nontoxic, antiulcer remedies derived from medicinal plants to treat NSAID-induced gastric ulcers (GU).

Aim: This study is a comparative assessment of the possible gastro-protective role of *Emblica officinalis* fruit pulp extract (EOFPE) and Omeprazole (Omepra) against Naproxin (Naprox)-induced GU.

Methodology: Five groups of rats (8 rats each) represent control, Naprox, Omepra (20 mg/kg), EOFPE (700 mg/kg), and EOFPE + Omepra. Omepra and EOFPE were ingested orally for 17 days. Naprox was ingested orally on day 15 for three consecutive days.

Results: Pretreatment with EOFPE and/or Omepra caused significant increases in gastric pH and gastric mucin content. Besides, significant decline in the total gastric acidity and gastric mucosal lesion relative to the Naprox group. Pretreatment with EOFPE and/or Omepra markedly improved the examined gastric pathologic features in Naprox-pretreated groups. Besides, EOFPE and/or Omepra overcome the gastric inflammation and oxidative stress markers induced by Naprox ingestion. Pretreatment with both EOFPE + Omepra leads to the best antiulcer impact and lesion inhibition percent relative to the Naprox groups pretreated with either EOFPE or Omepra alone.

Conclusion: EOFPE protected against Naprox-induced GU across anti-inflammatory and antioxidant mechanisms.

Keywords: Antioxidant, Anti-inflammatory, *Emblica officinalis*, Gastric ulcer.

INTRODUCTION

Gastrointestinal (GI) illnesses are among the most epidemic human illnesses worldwide[1]. Gastric ulcer (GU), a common GI disease, is ordinarily induced by several noxious factors like smoking, antiplatelet agents, and chronic usage of non-steroidal anti-inflammatory drugs (NSAIDs), anti-depressants, antibiotics, and antipsychotics drugs[2]. GU arises from an imbalance between gastric mucosa protective and invasive factors that induce mucosal protective barrier devastation and ulcer development. Oxidative stress, accumulation of reactive oxygen species (ROS), and rise formation of pro-inflammatory cytokines in the gastric mucosa are implicated in the induction of GU[3].

NSAIDs like Naproxen (Naprox), indomethacin, aspirin, and ibuprofen are extensively used to treat inflammation and manage pain. Frequent using of NSAIDs can trigger many adverse effects[4]. Continual administration of NSAIDs is highly liable to induce serious adverse severe effects like occult blood loss, increased hepatic enzymes, exacerbation of asthma, severe GI hemorrhage, and perforation from complicated complications ulcers[5].

As a standard model for NSAIDs, Naprox is a reversible inhibitor of the pro-inflammatory enzyme cyclooxygenase. It is commonly recommended in clinical practices for pain control in postoperative, post-traumatic, migraine, spinal pains, rheumatoid arthritis, and osteoarthritis. It is significantly effective in pain relief in the low therapeutic dose and long analgesic effect compared the ibuprofen. But similar to other NSAIDs, chronic usage of Naprox produced severe GI side effects. More attention is given to prevent and cure NSAIDs-induced complications, especially GU[6, 7].

Numerous synthetic drugs are prescribed for GU treatment. These drugs are expensive and confer simple to severe progressive adverse reactions in many cases. Subsequently, there is an urgent need to find an elective, non-toxic, and inexpensive alternative antiulcer therapy[8]. Today most people, in developed and developing countries depend on alternative medicine for preventing and curing several diseases. Therefore, scientists have been looking for new plants that possess antioxidant and anti-inflammatory activities and played an important role in various oxidative-stress-induced diseases[9].

Emblica officinalis (EO), commonly known as Amlaj, Amla, and Indian gooseberry, is a member of the Euphorbiaceae family. It is one of the myrobalans (plants with various therapeutic properties)[10]. EO fruit (EOF) is highly nutritious. It contains numerous vitamins, especially vitamin C and minerals[11]. Besides, it contains polyphenols, alkaloids, flavonoids, and phenolic compounds, like quercetin, emblicanin A, punigluconin, emblicanin B, and pedunculagin[12]. Extract of EOF showed a potent antioxidant, cytoprotective, hepatoprotective, anti-anticancer, hypolipidaemic, nephroprotective, and antivirus activities in earlier studies[13,14]. It has also been proved to exhibit anti-inflammatory and scavenger of hydroxyl and superoxide radicals[15]. EOF methanolic extract showed significant gastroprotective and healing effects in different acute gastric ulcer models (pyloric ligation, cold restraint stress, aspirin, and ethanol), as well as in chronic gastric ulcer (acetic acid)[16]. In addition, Al-Rehaily et al.[17] revealed

that EO extract showed antiulcer, anti-secretory, and cytoprotective activities in vivo models. A recent report also revealed the antiulcer activity of EO[18].

The present study is a comparative assessment of the potential gastro-protective role of EOFPE and Omepra, as a proton pump inhibitors drug, on Naprox-induced GU, focusing on its anti-inflammatory and antioxidant defense mechanisms.

MATERIALS AND METHODS

Drugs and kits: Gasec® tablets (40 mg Omepra) (Acino Pharma AG, Aesch, Switzerland) and Proxen® tablets (500 mg Naprox) (STADA Arzneimittel GmbH) were purchased from United Pharmacy and Al Dawaa Pharmacies, Jeddah, KSA, respectively. ELISA kits were obtained from Centronic Chemicals Co, Germany.

Fruit material and extraction: Fresh EOF was obtained from the local supermarket in Jeddah, KSA. EOF was authenticated in the Pharmaceutical Chemistry and Phytochemistry Department, Pharmacy College, KAU. The EOFP was dried then crushed to obtain a fine powder. 200 g dried powder of was soaked in l liter water-ethanol (1:1) for 18 h at room temperature with shaking to prepare EOFP 50 % aqueous-ethanolic extract. The collected supernatant was decanted, filtered, evaporated, lyophilized, and stored at -4 °C[19]. It yields 11.3 % of the dried fruits.

Naprox-induced ulcer in rats: All rats except the control have fasted for 18 hours before ingestion of the first dose of Naprox. Naprox (80 mg/kg) was administered at day 15 via gastric gavage twice daily for three consecutive days[20].

Rats and experimental protocol: Forty adult male rats (180-200 g) were obtained from the experimental house, King Fahd Medical Research Center (KFMRC), KAU. Rats handling was following the Canadian regulations established by KFMRC, KAU. Rats were acclimatized for 7 days in the standard animal lab/air-conditioned, fed ad libitum with free access to water. After the 7 days, rats were assigned into five groups (every 8 rats). Control, Naprox, Naprox + Omepra, Naprox + EOFPE, and Naprox + EOFPE + Omepra. Rats in the Control and Naprox groups were ingested orally distilled water for 17 days. Rats in the EOFPE and Omepra groups ingested orally EOFPE (700 mg/kg) and Omepra (20 mg/kg), respectively for 17 days[15, 21].

Stomach juice collection: All groups were sacrificed under anesthesia at day 17 (4 h after the last Naprox dose). The stomach was dissected out, opened along the greater curvature. Gastric content was collected and centrifuged. Total gastric acidity (mEq/L) and pH were measured[22]. Mucin (µg/ml) was determined in the collected gastric content samples[23]. Stomach specimens were washed with ice-cold saline, and then samples were either homogenized, centrifuged at 3000 rpm for 20 min then frozen at -20 °C for biochemical assay, or fixed in 10 % formalin for histopathological and histochemical examinations.

Evaluation of gastric mucosal lesions: All stomachs were macroscopically examined for measured length and width (10 x 10 mm^2) of hemorrhagic lesions with a planimeter. The ulcerated area was calculated by image processing software Image J. The total area of mucosal lesion was calculated as a percentage of the estimated mm^2 of the total ulcer area (TUA). The lesion inhibition percentage was calculated using the following equation:

Lesion inhibition (%) = [(TUA Naprox - TUA treated)/TUA Naprox] x 100

Histopathological and histochemical examination of gastric mucosa: Stomachs were sectioned by microtome (Leica, Germany). Sections were either stained with hematoxylin and eosin (H&E) for histopathological study of the changes induced in the structure of the gastric mucosa or stained with periodic acid Schiff (PAS) to differentiate the acidic and basic glycoproteins level in the mucus. Slides were examined under a light microscope (Olympus BX61-USA). The photographs were taken by a camera (Olympus DP72- USA) in the microscope unit at KFMRC.

Evaluation of gastric mucosal pro-inflammatory cytokines contents: According to the manufacturer's procedure, the tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β) concentrations were assessed in gastric tissues using ELISA kits.

Evaluation of gastric mucosal oxidants / antioxidants contents: According the manufacturer's procedure superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA) contents were assessed in gastric tissues using ELISA kits.

Statistical calculations: Results analysis was done using SPSS version 27. All results were exhibited as mean ± standard deviation, $p \leq 0.05$ was considered to forecast statistical significance.

RESULTS

Gastric pH and total acidity: A significant decline ($p \leq 0.001$) in gastric juice pH with a significant rise ($p \leq 0.001$) in total gastric acidity were observed in the Naprox group relative to the control group. Pretreatment with EOFPE and/or Omepra caused a significant ($p \leq 0.001$) rise in the gastric pH with a significant decline in the total gastric acidity ($p \leq 0.001$) relative to the Naprox group. Pretreatment with EOFPE + Omepra leads to noticeable improved in gastric juice pH and total gastric acidity. There was a significant ($p \leq 0.01$) difference between the group pretreated with EOFPE + Omepra relative to the group pretreated with EOFPE alone in gastric juice pH and total acidity. No significant difference in gastric pH and total acidity was noticed between groups pretreated with EOFPE or Omepra before -Naprox ingestion (Table 1).

Table 1: The effects of pretreatment with EOFPE, Omepra, and their combination on gastric pH and total acidity measured in rats.

Groups	Gastric pH	Total gastric acidity (mEq/L)
Control	3.91 ± 0.27	63.3 ± 5.67
Naprox	2.33 ± 0.19 $^{\$***}$	98.01 ± 6.30 $^{\$***}$
Naprox + Omepra	3.62 ± 0.35 $^{\#***}$	64.77 ± 3.41 $^{\#***}$
Naprox + EOFPE	3.34 ± 0.49 $^{\#***}$	74.51 ± 3.61 $^{\#***}$
Naprox + EOFPE+ Omepra	3.89 ± 0.22 $^{\#***, \&**}$	61.45 ± 3.29 $^{\#***, \&**}$

The values are mean ± SD (n=8). $^{\$}$Significant differ than Control; $^{\#}$differ than Naprox; $^{\&}$differ than Naprox + EOFPE. ($^{**}p \leq 0.01$, $^{***}p \leq 0.001$).

Gastric mucin content, total ulcer area (TUA), and lesion inhibition (%) : Ingestion of Naprox resulted in a significant decline ($p \leq 0.001$) in mucin content relative to

the control group. Pretreatment with EOFPE and/or Omepra caused a significant ($p \leq 0.001$) rise in mucin content relative to the Naprox group. Pretreatment with EOFPE + Omepra improved gastric mucin secretion. There was a significant ($p \leq 0.01$) change between the group pretreated with EOFPE+ Omepra relative to the group pretreated with EOFPE alone. No significant difference in gastric mucin content was noticed between groups pretreated with EOFPE or Omepra pre-Naprox ingestion (Table 2).

As shown in Table 2, Naprox ingestion resulted in a significant increase in TUA (25.68 ± 4.13) ($p \leq 0.001$) relative to the control rats. On the other hand, marked decline ($p \leq 0.001$) in TUA was shown in groups pretreated with either EOFPE, Omepra, or their combination relative to the Naprox group. Ompera caused 79.98% inhibition in lesion formation while EOFPE caused 70.95% inhibition. Pretreatment with EOFPE + Omepra leads to the best impact on the TUA (2.84 ± 0.61) and the highest lesion inhibition (88.94 ± 5.23 %). Pretreatment with Omepra revealed a significant decrease in TUA with a significant increase in lesion inhibition % compared to the group pretreated with EOFPE ($p \leq 0.05$). In addition, pretreatment with EOFPE + Omepra induced significantly decreased TUA and increased lesion inhibition % relative to groups pretreated with either EOFPE or Omepra alone ($p \leq 0.01$ and $p \leq 0.05$, respectively).

Table 2: The effects of pretreatment with EOFPE, Omepra, and their combination on gastric juice mucin content, total ulcer area (TUA) and lesion inhibition % (LI %) measured in rats.

Groups	Mucin (µg/ml)	TUA (mm^2)	LI %
Control	356.11 ± 25.73	-	-
Naprox	206.12 ± 27.39$^{\$***}$	25.68 ± 4.13$^{\$***}$	-
Naprox + Omepra	339.61 ± 18.07$^{\#***}$	5.14 ± 0.84$^{\#***}$	79.98 ± 9.37$^{\#***}$
Naprox + EOFPE	321.82 ± 20.73$^{\#***}$	7.46 ± 0.81$^{\#***,@*}$	70.95 ± 8.55$^{\#***,@*}$
Naprox + EOFPE + Omepra	359.79 ± 13.09$^{\#***,\&**}$	2.84 ± 0.61$^{\#***,@*,\&**}$	88.94 ± 5.23$^{\#***,@*,\&**}$

The values are mean ± SD (n=8). $^{\$}$ Significant differ than Control; $^{\#}$ differ than Naprox; $^{@}$ differ than Naprox + Omepra, $^{\&}$ differ than Naprox + EOFPE. (* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$).

Gastric histopathological alterations (H & E Stain): Sections from the control group showing normal structures (Figure 1 A & B). Sections from the Naprox group pointed out characteristic histopathological features of GU (Figure 1 C & D). Sections from the Naprox + Omepra group revealed apparently normal histopathological structures. However, a few sections showed remnants of regenerating erosive lesions with minimal tissue destruction. A few submucosa capillaries showed mildly dilated (Figure 1 E & F). Sections from the Naprox + EOFPE group revealed apparently normal. However, a few sections showed remnants of regenerating erosive lesions with minimal tissue destruction, and a few gastric glands appeared mildly dilated. The submucosa showed mild oedematous and inflammatory reaction (Figure 1 G & H). The gastric sections from the Naprox + EOFPE + Omepra group revealed a healing process in the mucosal covering epithelium The underlying glandular epithelium of the different types appeared normal (Figure 1 I & J).

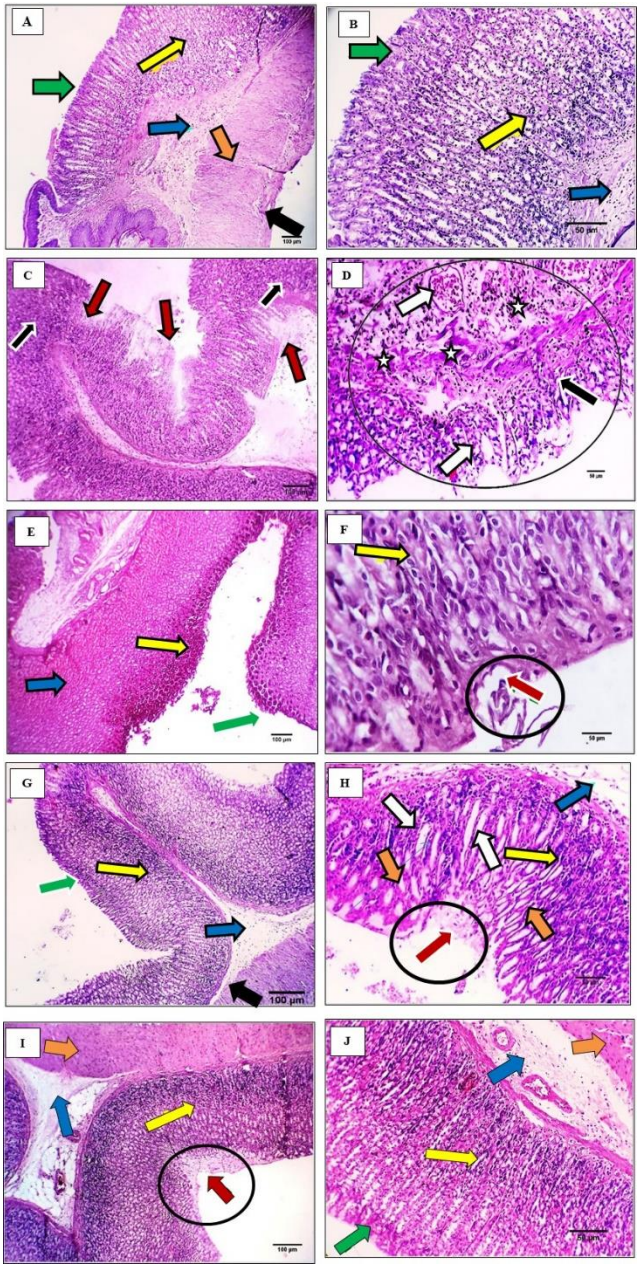

Figure 1: Gastric tissue histopathology associated with Naprox-induced ulcer in rats (H & E stain, bars 100 and 50 µm). Photos (A & B) showed gastric sections of the control rats, photos (C & D) showed gastric sections of the Naprox rats, photos (E & F) showed gastric sections of Naprox + Omepra rats, photos (G & H) showed gastric sections of Naprox + EOFPE rats, and photos (I & J) showed gastric sections of the Naprox + EOFPE + Omepra rats.

Gastric histochemical findings (PAS stain): Examined sections from gastric mucosa, submucosa, muscular coat, and serosa of the control group stained with PAS revealed moderate reactivity of the mucosal and glandular cells.

Positive cells showed magenta red coarse eosinophilic cytoplasmic granular materials (glycoproteins) (Figure 2 A). In the Naprox group, gastric sections revealed negative reactivity of the destructed mucosal lining and underlying glandular cells (0/4 of the mucosal thickness) (Figure 2 B). Gastric sections of the Naprox + Omepra, Naprox + EOFPE, and Naprox + EOFPE + Omepra groups revealed marked reactivity of the regenerated mucosal lining and underlying glandular cells (1.30-1.40/4; 1.25-1.30/4 and 1.35-1.45/4, respectively of the mucosal thickness in the erosive or ulcerative areas which underwent the regenerative change), other parts of the gastric mucosa showed reactivity comparable to that of the control group (Figures 2 C, D & E, respectively).

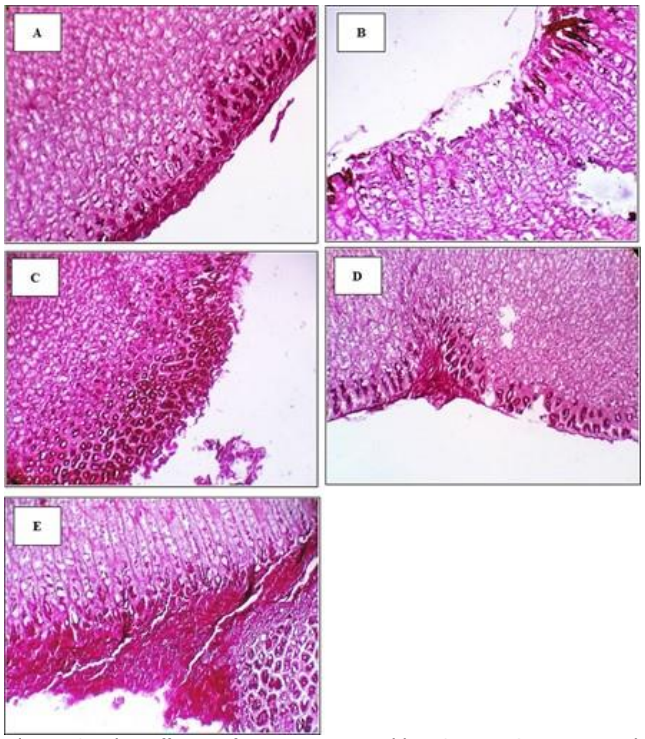

Figure 2: The effects of pretreatment with EOFPE, Omepra, and their combination on gastric histochemical findings (PAS stain, bar 50 µm). Photo (A) section of the control, photo (B) section of the Naprox rats, photo (C) section of Naprox + Omepra rats, photo (D) section of Naprox + EOFPE rats, and photo (E) section of Naprox + EOFPE + Omepra rats.

Gadtric inflammatory indicators (TNF-α and IL-1β): As shown in Figure 3, Naprox ingestion resulted in gastric inflammatory response which is evidenced by significant ($p \leq 0.001$) rises in gastric TNF-α and IL-1β concentrations relative to the control rats. Pretreatment with EOFPE and/or Omepra caused significant ($p \leq 0.001$) decreases in gastric TNF-α and IL-1β concentrations relative to the Naprox group. Pretreatment with EOFPE + Omepra leads to a noticeable decline in gastric TNF-α and IL-1β concentrations. There were significant ($p \leq 0.05$) difference in gastric TNF-α and IL-1β concentrations between the group pretreated with EOFPE+ Omepra and the group pretreated with EOFPE alone. No significant difference was noticed between groups pretreated with EOFPE or Omepra before-Naprox ingestion.

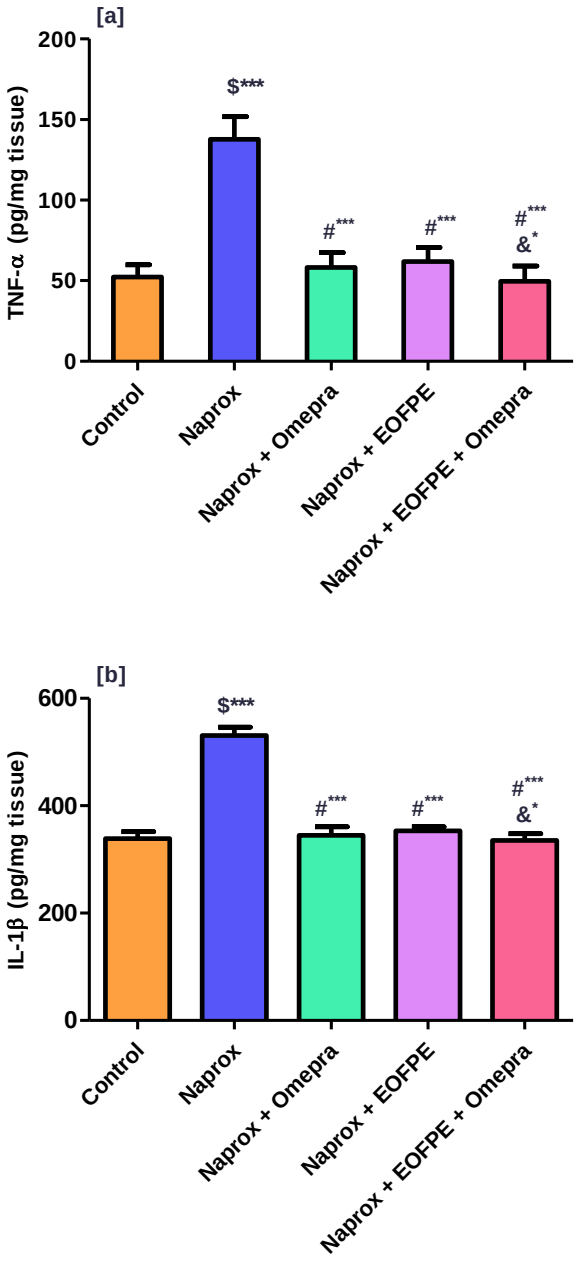

Figure 3: The effects of pretreatment with EOFPE, Omepra, and their combination on stomach mucosa contents of inflammatory indicators (TNF-α [a] and IL-1β [b]) measured in rats. The values are mean ± SD (n=8). $ Significant differ than Control; # differ than Naprox; & differ than Naprox + EOFPE. (* $p \leq 0.05$, *** $p \leq 0.001$).

Gastric antioxidant indicators (SOD, CAT, and MDA): As shown in Table 3, Naprox ingestion induced significant ($p \leq 0.001$) decline in gastric SOD and CAT activities with a significant ($p \leq 0.001$) elevation in the gastric MDA concentration relative to the control rats. Pretreatment with EOFPE and/or Omepra caused significant ($p \leq 0.001$)

increases in gastric antioxidant SOD and CAT activities with a significant ($p \leq 0.001$) decrease in gastric MDA concentration relative to the Naprox group. Pretreatment with both EOFPE + Omepra leads to noticeable neutralize the depletion in the gastric enzymatic antioxidant activities. There was significant ($p \leq 0.05$) alteration between the group pretreated with both EOFPE + Omepra relative to groups pretreated with either EOFPE or Omepra alone. No significant difference in gastric SOD, CAT, and MDA concentrations was noticed between groups treated with EOFPE or Omepra before-Naprox ingestion.

Table 3: The effects of pretreatment with EOFPE, Omepra, and their combination on stomach mucosa contents of antioxidant indicators (SOD, CAT, and MDA) measured in rats.

Groups	SOD (U/mg tissue)	CAT (U/mg tissue)	MDA (nmol/mg tissue)
Control	58.21 ± 7.96	15.19 ± 2.60	12.34 ± 2.12
Naprox	27.63 ± 5.15$^{\$***}$	6.08 ± 1.23 $^{\$***}$	23.53 ± 4.35 $^{\$***}$
Naprox + Omepra	48.41 ± 4.42$^{\#***}$	11.75 ± 1.86 $^{\#***}$	15.39 ± 3.15 $^{\#***}$
Naprox + EOFPE	50.26 ± 7.34$^{\#***}$	12.36 ± 1.76$^{\#***}$	14.46 ± 2.71 $^{\#***}$
Naprox + EOFPE+ Omepra	56.58 ± 4.15$^{\#***, @*, \&*}$	14.34 ± 1.74 $^{\#***, @*, \&*}$	11.33 ± 2.30 $^{\#***, @*, \&*}$

The values are mean ± SD (n=8). $^{\$}$ Significant differ than Control; $^{\#}$ differ than Naprox; $^{@}$ differ than Naprox + Omepra; $^{\&}$ differ than Naprox + EOFPE. (* $p \leq 0.05$, *** $p \leq 0.001$).

DISCUSSION

In fact, NSAIDs continue to be the leading drug for a variety of illnesses. On the other hand, they have been reported to cause oxidative stress, which is associated with the etiology of gastric mucosal destruction[24]. The current research utilized a rat Naprox-induced gastric ulcer model. This model was chosen because Naprox is the most commonly consumed NSAID. Naprox also causes pyloric gastric ulcers modeled on human gastric ulcers[20]. Herbal remedies have recently been shown to be promising in the fight against gastric ulcer[25].

Similar to the current study results, oral gavage of the butanol extract of EOF (100 mg/kg) to rats enhanced gastric mucus secretion in the indomethacin-induced ulceration. The macroscopic appearance of the stomach also showed a protective effect of the extract on the gastric wall against indomethacin-induced lesions. The extract also reduced MDA and increased gastric contents of SOD compared to the ulcer group[26]. Furthermore, EOFPE (60 mg / kg) was found to have a significant healing effect on indomethacin-induced gastric ulcer and a high healing rate on day seven. MDA and protein carbonyl concentrations were lowered by EOFPE treatment at a 60 mg/kg dose, after which the total thiol content and serum total antioxidants level were significantly increased[27].Antioxidants have been shown to help heal NSAIDs-induced gastric ulcers, as the generation of NSAID-induced gastric lesions involves oxidative cell damage[28]. Ethanolic extract of EO fruits was found to have the highest antioxidant activity compared to other solvent extracts, as determined by the DPPH scavenging assay[27]. In agreement of the present study results, the ethanol extract of EO fruits reduced levels of pro-inflammatory cytokines. It lowers inflammatory cytokines (TNF-α and IL1β) and at the same time induces IL-10 levels in tissues for a healing effect[27]. The generation of inflammatory cytokines is a critical element in developing gastric mucosal damage. Increased expression of pro-inflammatory cytokines (IL-1β and TNF-α) and a reduction in anti-inflammatory cytokines (IL-10) at the mucosal level is one of the most prominent ways of mediating NSAID-induced gastropathy. This resulted in a cytokine imbalance linked to the severity of ulceration[29]. Polyphenolic compounds such as gallic acid and ellagic acid (ellag) and a high quantity of ascorbic acid are known to be vital antioxidant elements of EO extract. EO extract is an excellent antioxidant because it removes superoxide radicals and preserves antioxidant enzymes such as SOD required for biosafety[12]. Ellag therapy protected the stomach mucosa from aspirin-induced ulcer. It significantly decreased aspirin-induced rise in gastric MDA contents. It also significantly decreased the markers of inflammation including IL-6 and myeloperoxidase (MPO)[30]. Ellag's ability to inhibit neutrophil infiltration allowed it to promote healing of NSAID-induced stomach ulcers in mice. The modification of the COX-pathway by ellag aids in enhancing mucosal growth factors and maintain a balance of pro-anti-inflammatory cytokines to promote ulcer healing[31]. Besides, gallic acid produced a protective impact against ethanol-induced stomach ulcers in rats. The underlying mechanism of gallic acid gastric preservative action might be involved antioxidant[3]. Gallic acid was also found to inhibit ulcer formation in aspirin and pyloric ligation ulcer models by increasing SOD, CAT, and GSH levels in the rat gastric mucosa and reducing MPO and MDA levels[32].

Proton pump inhibitors (as Omepra) suppress gastric acid output in a significant and lengthy manner and effectively heal NSAID-related ulcers, particularly those exposed to NSAIDs for a long time [33]. In this study, Omepra provided protection against Naprox-induced gastric ulcer comparable to EOFPE. Adding Omepra to EOFPE had a more pronounced effect compared to EOFPE alone.

CONCLUSION

The current results showed the protective effect of EOFPE on Napro-induced gastric ulcer in rats. This is reflected in the preserved acidity of the stomach and the maintenance of mucus secretion and gastric tissue structure. Antioxidant and anti-inflammatory mechanisms are the basis of the protective effect of EOFPE in this model. The extract can be used as a prophylactic adjuvant therapy with Omepra to prevent gastric ulcer.

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