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Original Research Article

Costus afer (Costaceae, Zingiberales) leaf extract ameliorates naproxen-induced gastric ulcer in rats

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Abstract

Purpose: *To examine Costus afer leaf extract (CALE) protective effects against naproxen-induced gastric ulcer and the mechanisms of protection.*
Methods: *Rats with naproxen-induced ulcer were pretreated with either CALE (800 mg/kg), pantoprazole (20 mg/kg), or a combination of both. Then the ulcer index, total gastric acidity, gastric pH, and curative index were evaluated. In addition, gastric mucin, pepsin, prostaglandin E2, nitric oxide, reduced glutathione, lipid peroxide, and superoxide dismutase were quantified. The gastric pathological change was also evaluated.*
Results: *Rats treated with CALE, pantoprazole, and their combination significantly decreased ulcer index, total gastric acidity, and gastric pH. All treatments induced a significant curative index in favor of the combination. The CALE significantly increased gastric mucin, prostaglandin E2, nitric oxide, reduced glutathione, and superoxide dismutase. However, the CALE significantly decreased pepsin and lipid peroxide product.*
Conclusion: *These results reveal that CALE protects the stomach against naproxen-induced ulcer. This action is linked to increased gastroprotective factors, increased antioxidants, and decreased lipid peroxidation. The CALE may be used as an adjunctive treatment for ulcers caused by NSAIDs.*

Keywords: *Costus afer, Naproxen, Gastric ulcer, Antioxidants, Lipid peroxidation*

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INTRODUCTION

Gastric ulcer is one of the most common gastrointestinal disorders that affect many people around the world [1]. It induced *by* disequilibrium between gastric protective factors (antioxidant, anti-inflammatory, mucus, mucin, nitric oxide, and prostaglandins) and invasive factors (stress and nonsteroidal anti-inflammatory drugs (NSAID) [2]. Naproxen is a commonly used NSAID in arthritic patients. It possesses anti-inflammatory, antipyretic, and analgesic properties [3]. Antral ulcers, erosions, and petechial bleeding in the gastrointestinal tract are the most common adverse effects associated with naproxen. The suggested mechanism(s) of naproxen-induced gastrointestinal ulceration were the generation of lipid peroxides and oxygen free radicals [4].

Most of the antiulcer drugs generate many undesirable adverse reactions such as abdominal pain, dizziness, diarrhea, constipation, headache, and bowel upset. Recently, natural plants have attracted scientific attention globally as prophylactic alternatives for many diseases [5]. *Costus afer* (family Costaceae), commonly called bush cane or ginger lily, is a famous medicinal herb that possesses antioxidant properties [6]. It contains numerous antioxidant bioactive constituents as phenols, flavonoids, alkaloids, terpenoids, and sterols [7].

Several studies have shown that *Costus afer* leaf extract (CALE) exhibits immunomodulatory, hypoglycemic, antitussive and antimalarial effects [7,8]. The plant extract treats tachycardia and respiratory problems [7]. Moreover, CALE exerts a cardioprotective effect against carbon tetrachloride-induced cardiotoxicity [9]. It is used as a remedy to treat inflammatory disorders and arthritis [10]. The extract of *Costus speciosus* rhizome has proven to heal ulcers in rats [11].

So far, no studies have reported the antiulcer potential of CALE. Therefore, this study aimed to assess the potential antiulcer effect of CALE against naproxen-induced gastric ulcer in rats.

EXPERIMENTAL

Drug and chemicals

Proxen (500 mg/tablet naproxen Grunenthal GmbH, Germany) and pantozol (20 mg/tablet pantoprazole Takeda GmbH, Germany) were purchased from Nahdi Pharmacy, Jeddah, Saudi Arabia (SA).

Preparation of CALE

Costus afer leaf was purchased from Abazeer Organic Store, Jeddah, SA. Prof. Alaa Eldin M.S. Khedr at the Department of Pharmaceutical Chemistry and Phytochemistry, College of Pharmacy, King Abdulaziz University, Jeddah, SA has authenticated the *Costus afer* leaf and a voucher specimen (CAL-360) was kept at this department. The milled dried CAL (500 g) was soaked in 1.5 L 80% ethyl alcohol. The solution was stirred at 100 rpm for 48 h at 25 °C. The filtrate was evaporated at 40 °C under vacuum. The extract was then freeze-dried by a Freeze-Dryer Lyophilizer, Virtis, USA and kept at 4 °C [8].

Determination of bioactive compounds

The bioactive compounds of CALE were assayed [12].

Animals

Fifty male adult albino rats (200 - 220 g weight) were acquired from King Fahd Medical Research Center, Jeddah, SA. The rats were housed in the standard environment of air, temperature, and humidity. The experimental work was performed following guidelines established by the International Standard and Institutional Animal Care and Use committee (IACUC) [13]. The animal work was approved by the Biomedical Ethics Committee, King Fahd Medical Research Center, Jeddah, SA (approval no. 162060074).

Induction of gastric ulcer

Peptic ulcer was induced in rats by ingestion of 80 mg/kg naproxen twice daily for 3 days after fasting the rats for 18 h before the first dose of naproxen [14].

Treatment protocol

The rats were divided into 5 groups (n = 10). 1- Control: rats treated with oral distilled water twice-daily for 17 days. 2- Ulcer: rats treated with oral naproxen 80 mg/kg twice-daily for three days. 3- CALE: rats treated with oral 800 mg/kg CALE [9]. 4- Pantoprazole: rats treated with oral 20 mg/kg pantoprazole [15]. 5- CALE + Pantoprazole: rats orally treated with both CALE and pantoprazole. The rats in groups 3-5 were treated with either CALE, pantoprazole or their combination for 14 days before ulcer induction and for 3 days thereafter. Four hours post the last naproxen dose the stomachs were dissected, and the gastric juice was collected. The gastric lesions were counted using a magnifying lens and the ulcer index (UI) (mm^2) was calculated [16]. The curative index (CI) was calculated using the following equation:

Curative index (CI) = ((UI of Ulcer group – UI of Treated group)/UI of Ulcer group) x 100

Each stomach was divided into two parts: one part was frozen at -80 °C for biochemical analysis while the other part was fixed in 10 % buffered formalin for the histopathological examination.

Determination of total gastric acidity and pH

The stomach was extracted and opened from the large curvature, the stomach contents were withdrawn into a tube, diluted with water, a centrifuge was made at 3000 rpm for 10 min, and the clear supernatant was separated. The total gastric acidity in mEq/L was measured [17]. The

pH of the gastric juice was measured using a pH meter.

Determination of gastric mucin and pepsin contents

Pepsin was determined using Folin's reagent and then absorbance readout at 660 nm. Mucin was determined using alcian blue dye and $MgCl_2$ and then absorbance readout at 605 nm [18].

Histopathological examination

The formalin-fixed stomach tissues were dehydrated in graded alcohol, cleared in xylene, embedded in paraffin, cut into 3 - 5 µm thick sections, stained with hematoxylin and eosin (H & E) and examined under light microscope.

Determination of gastric antioxidant and anti-inflammatory measures

Gastric mucosal lipid peroxidation measured as malondialdehyde (MDA), nitric oxide (NO), reduced glutathione (GSH), superoxide dismutase (SOD), and prostaglandin E2 (PGE2) were measured using ELISA assay kits (Glory Science Co., Ltd. Del Rio-TX-USA).

Statistical analysis

All statistics were performed using SPSS software, version 24. Data are presented as mean ± SD. Statistical significance between groups were assessed using ANOVA test and $p < 0.05$ was considered significant.

RESULTS

Bioactive compounds of CALE

CALE possessed a considerable quantity of terpenoids, glycosides, phenols, and flavonoids. Sterols, alkaloids, and saponins were found in lower quantities. Besides, smaller amounts of tannins were also found (Figure 1).

Effect of CALE on UI, total gastric acidity, pepsin, gastric pH, and CI

Naproxen administration in rats significantly increased UI, total gastric acidity, and pepsin relative to the control results. CALE, pantoprazole, and their combination reduced UI, total gastric acidity, and pepsin significantly relative to ulcer results. Pantoprazole reduced UI and total gastric acidity significantly relative to CALE results. In addition, combination of CALE and pantoprazole reduced UI, total gastric acidity, and pepsin significantly relative to pantoprazole results (Figure 2 A-C).

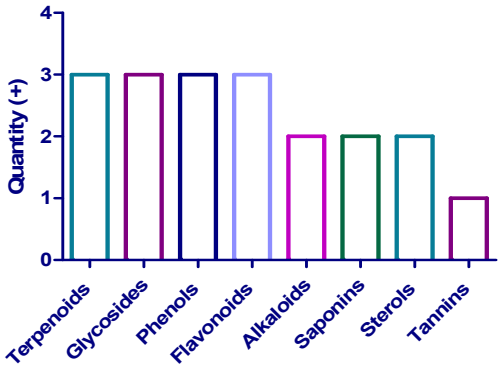

Figure 1: Bioactive compounds found in the extract of CALE

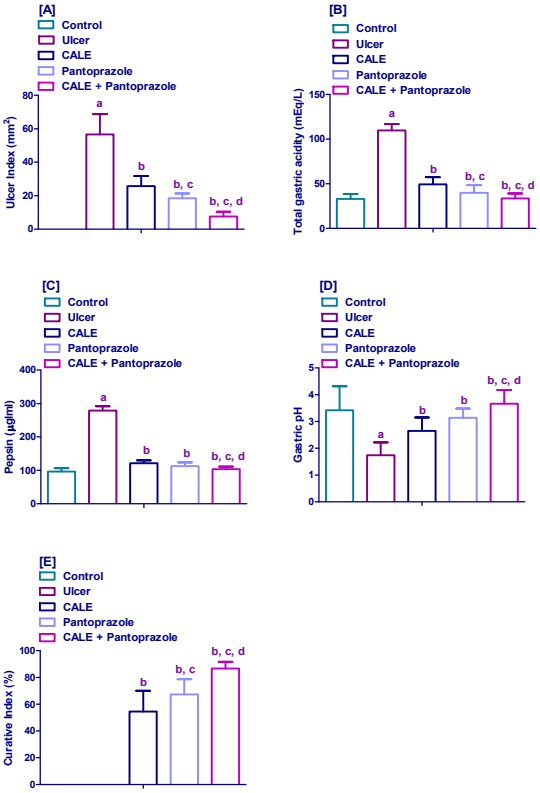

Figure 2: Effect of CALE and/or pantoprazole on A: Ulcer index (UI), B: Total gastric acidity, C: Pepcin, D: Gastric pH, and E: Curative index (CI). Values are presented as mean ± SD (n=10). [a] Significantly different from Control group; [b] significant difference from Ulcer group; [c] significant difference from CALE group; [d] significantly different from Pantoprazole group. ($p < 0.05$)

Naproxen administration significantly decreased gastric pH relative to the control results. CALE, pantoprazole, and their combination significantly

increased gastric pH compared to ulcer results. In addition, the combination of CALE and pantoprazole significantly increased gastric pH compared to pantoprazole results (Figure 2 D). Regarding the gastric pH, an equal effect was obtained from the CALE and pantoprazole. Whereas, there was an improved therapeutic effect of the combination.

CALE administration produced 54.54% CI, but the CI of the pantoprazole was still exceeded (67.34%). Whereas, there was an improved CI of the combination (86.69%) (Figure 2 E).

Impact of CALE on gastric mucosal histopathology

Low-power photos revealed that the control stomach wall was of normal mucosa, normal submucosa, and normal musculosa (Figure 3A). The stomach of the ulcer group showed 3 deep ulcers, damaged muscularis mucosa, and marked submucosal edema (Figure 3B). The stomach of CALE treated group showed two superficial ulcers which were not reaching muscularis mucosa, mild submucosal edema with mildly dilated and congested blood vessels (Figure 3C). The stomach of the pantoprazole treated group showed multiple superficial ulcers which were not reaching muscularis mucosa, and mild submucosal edema (Figure 3D). The stomach of the CALE and pantoprazole combination treated group showed one superficial ulcer which was not reaching muscularis mucosa, and mild submucosal edema (Figure 3E).

The high-power photos revealed that the control stomach wall was of healthy parietal cells, normal chief cells, muscularis mucosa, and submucosa (Figure 4 A). The stomach of the ulcer group showed ulcers with complete damage of muscularis mucosa, excess parietal cells, and few chief cells (Figure 4 B). The stomach of CALE treated group showed superficial ulcers with mild inflammatory infiltrate, average parietal, and chief cells (Figure 4C). The stomach of the pantoprazole treated group showed superficial ulcers which were not reaching muscularis mucosa, less parietal cells, mild submucosal edema, and mild inflammatory infiltrate (Figure 4 D). The stomach of the CALE and pantoprazole combination treated group showed normal mucosa with an intact superficial layer and normal glands (Figure 4E).

The pathological examination showed a significant improvement in the combination group compared to both treatments alone.

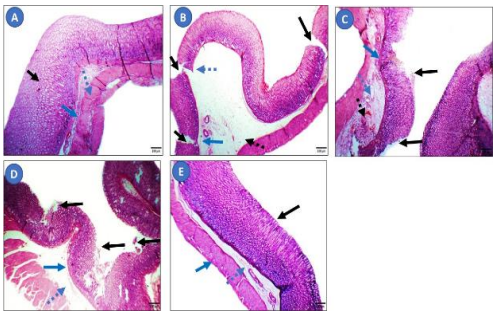

Figure 3: Impact of CALE and/or pantoprazole on gastric mucosa histopathology (H & E, x 100). **Control (A):** Stomach wall showing normal mucosa (black arrow), submucosa (blue arrow) and mucosa (blue dotted arrow). **Ulcer (B):** Stomach wall showing 3 ulcers (black arrows), deep (blue arrow), damaged muscularis mucosa (blue dotted arrow) and marked submucosal edema (black dotted arrow). **CALE (C):** Stomach wall showing 2 superficial ulcers (black arrows) not reaching muscularis mucosa (blue arrow), mild submucosal edema (blue dotted arrow), mild dilated and congested blood vessels (black dotted arrow). **Pantoprazole (D):** Stomach wall showing multiple superficial ulcers (black arrows) not reaching muscularis mucosa (blue arrow), and mild submucosal edema (blue arrow). **CALE + Pantoprazole (E):** Stomach wall showing 1 superficial ulcer (black arrow) not reaching muscularis mucosa (blue arrow), and mild submucosal edema (blue dotted arrow)

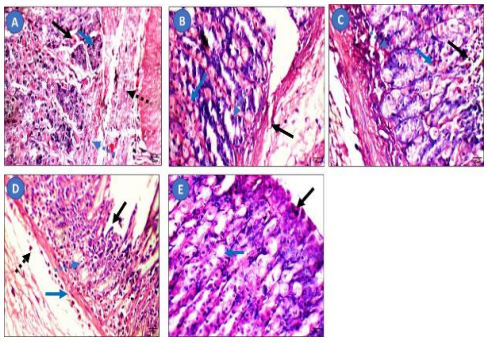

Figure 4: Impact of CALE and/or pantoprazole on gastric mucosa histopathology (H & E x 400). **Control (A):** The stomach wall showed normal parietal cells (black arrow), chief cells (blue arrow), muscularis mucosa (blue dotted arrow), and submucosa (black dotted arrow). **Ulcer (B):** The stomach wall showed ulcers with complete destruction of muscularis mucosa (black arrow), excess parietal cells (blue arrow) and few chief cells (blue dotted arrow). **CALE (C):** The stomach wall showed base of superficial ulcers, mild inflammatory infiltrate (black arrow), average parietal (blue arrows) and chief cells (blue dotted arrow). **Pantoprazole (D):** The stomach wall showed superficial ulcer (black arrow) not reaching muscularis mucosa (blue arrow) with reduction of parietal cells (blue dotted arrow), mild submucosal edema, and mild inflammatory infiltrate (black dotted arrow). **CALE + Pantoprazole (E):** The stomach wall showed normal mucosa with intact superficial layer (black arrow) and normal glands (blue arrow)

Effect of CALE on gastric mucosal protective factors

Naproxen administration in rats significantly decreased mucin, PGE2, and NO relative to the control results. Treatment of ulcer induced rats with CALE, pantoprazole, and their combination significantly increased mucin, PGE2, and NO compared to ulcer results. There was no significant difference between pantoprazole results and CALE results. However, the combination of CALE and pantoprazole significantly increased mucin and PGE2 when compared to CALE results. In addition, the combination of CALE and pantoprazole significantly increased mucin, PGE2, and NO when compared to pantoprazole results (Figures 5 A-C).

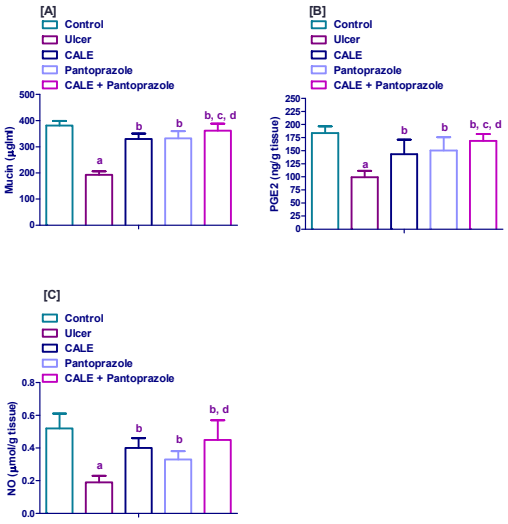

Figure 5: Effect of CALE and/or pantoprazole on A: Mucin, B: Prostaglandine E2 (PGE2), and C: Nitric oxide (NO). Values are presented as mean ± SD (n=10). [a] Significantly different from Control group; [b] significantly different from Ulcer group; [c] significant difference from CALE group; [d] significantly different from Pantoprazole group ($p < 0.05$)

Effect of CALE on gastric mucosal oxidative stress measures

Naproxen administration in rats significantly increased gastric MDA relative to the control value. CALE, pantoprazole, and their combination significantly decreased gastric MDA when compared to ulcer results. Treatment of ulcer induced rats with CALE significantly decreased gastric MDA when compared to pantoprazole results. In addition, the combination of CALE and pantoprazole significantly decreased gastric MDA compared to pantoprazole results (Figure 6A).

On the other hand, naproxen administration in rats significantly decreased both gastric GSH and SOD relative to the control results. CALE, pantoprazole, and their combination significantly increased both gastric GSH and SOD when compared to ulcer results. Treatment of ulcer induced rats with CALE significantly increased both gastric GSH and SOD when compared to pantoprazole results. In addition, the combination of CALE and pantoprazole significantly increased both gastric GSH and SOD compared to pantoprazole results (Figure 6B and C).

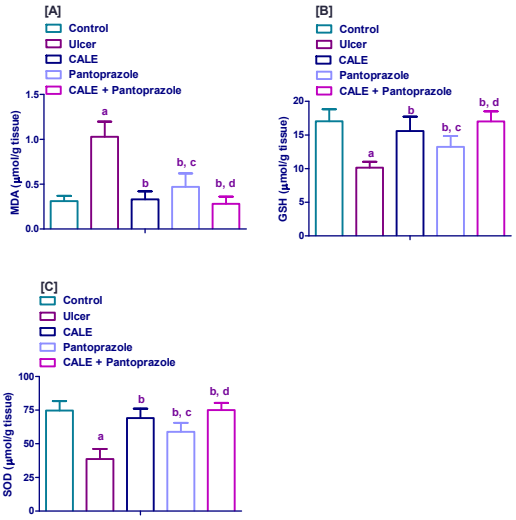

Figure 6: Effect of CALE and/or pantoprazole on A: Lipid peroxide (MDA), B: Reduced glutathione (GSH), and C: Superoxide dismutase (SOD). Values are presented as mean ± SD (n=10). [a] Significantly different from control group; [b] significantly different from Ulcer group; [c] significant difference from CALE group; [d] significantly different from Pantoprazole group ($p < 0.05$)

DISCUSSION

This study is aimed to confirm the potential protective effect of CALE against experimentally induced gastric ulcer with naproxen relative to the effect of pantoprazole. The potential for a more effective therapeutic impact was also investigated by adding CALE to pantoprazole as an adjuvant remedy in the treatment of naproxen-induced gastric ulcer. From the results of this study, CALE showed a significant protective effect against naproxen-induced stomach ulcers. This was evident from the decrease in the ulcer index, total gastric acidity, and pepsin secretion. It was also found that the extract protected the pH of the stomach juice. Pantoprazole outperformed the extract in reducing ulcer index as well as total gastric acidity while CALE and pantoprazole produced

equal therapeutic effect against pepsin and gastric pH. The therapeutic impacts of the combination exceeded that of each drug alone. Moreover, the histopathological results supported these results.

In folk medicine, infusion of *Costus afer* flowers and rhizomes have been utilized to manage stomach illness [11]. Usually, the majority of NSAIDs produce stomach ulcer in the corpus area in the form of erosions, not ulcers. However, in humans, the ulcers accompanied using NSAID were localized in the gastric antrum. Naproxen has been reported to induce stomach ulcer in the antrum region [4]. Due to this similarity, this model was used in the present study. This study is the first to report the protective effect of CALE against stomach ulcer caused by naproxen in rats. There is only one recently published study on another *Costus species* (*Costus speciosus*) that reported its effectiveness against stomach ulcer in rats. In the pylorus ligation model of stomach ulcer, administration of the hydroalcoholic extract of *Costus speciosus* rhizome markedly reduced the gastric total and free acidity, and markedly improved the gastric pH, which suggested that the extract exerted an antisecretory effect. It also appeared that feeding the extract at the higher dose (400 mg/kg) helped to heal the ulcers [11].

Costus afer leaf extract used either alone or in combination with pantoprazole, significantly increased gastric mucosal mucin, prostaglanidin E2 (PGE2), and nitric oxide (NO). Nitric oxide is an endogenous protective factor that safeguards the stomach wall [19]. It is known to protect the gastric epithelium and the mucus membrane health. As a vasodilator, NO promotes healthy stomach blood supply [20]. PGE2 is the master element that controls the ulcer healing. PGE2 triggers bicarbonate and mucus production, regulates the mucosal turnover, and preserves mucosal blood supply [21]. The ulcerogenic potential of naproxen was based on its non-selective inhibition of both cyclooxygenase (COX1 and COX2) that result in the inhibition of PG formation [22]. CALE protects the gastric mucosa against naproxen-induced stomach ulcer by promoting the secretion of gastric mucosal protective factors (mucin, PGE2, and NO). In agreement with this study results, it has been reported that NO donating naproxen formulation protected against L-NAME-induced gastric ulcers [23].

The results of this study showed that naproxen-induced gastric ulcer was accompanied by increased MDA and decreased GSH and SOD. Previous research has shown that naproxen-induced stomach ulcers was associated with oxidative stress environment, increased free radical formation, and lipid peroxidation. Besides, the gastric contents of antioxidant enzymes (SOD, CAT, and GPx) was significantly lowered post-naproxen ulceration. These findings suggested a direct relation that may link naproxen-induced ulceration and oxidative stress [4]. In this study, the administration of CALE decreased MDA and increased contents of GSH and SOD in the gastric mucosa. The results of this study showed that CALE contains large quantities of flavonoids and phenols, compounds known for their antioxidant effectiveness [7]. It has also been reported that the therapeutic effects of CALE may be linked to its enhancement of enzymatic and non-enzymatic antioxidants, as well as its ability to scavenge free radicals [9,24].

CONCLUSION

The findings of this study reveal that CALE protects against naproxen-induced gastric ulcer in rats. This action is linked to increased PGE2, NO, antioxidants (GSH & SOD), and decreased lipid peroxidation. In addition, a combination of CALE and pantoprazole showed a superior ulcer healing action relative to either CALE or pantoprazole. The CALE may be used as an adjunctive treatment for ulcers caused by NSAIDs.

DECLARATIONS

Acknowledgement

The authors gratefully acknowledge King Fahd Medical Research Center for help in conducting the practical part of this work at their animal house.

Conflict of interest

No conflict of interest is associated with this work.

Authors' contribution

The authors declare that this work was done by the author(s) named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Hala Khattab and Buthaina Aljehany conducted the experimental model and wrote the manuscript. Hala Khattab designed the protocol. Buthaina Aljehany performed statistical analysis.

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