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The role of nuclear factor kappa B signaling in the therapeutic effect of tadalafil against dexamethasone-induced gastric ulcer in rats

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ABSTRACT

Gastric ulceration is a common gastrointestinal ailment with serious consequences that can lead to serious illness or even death. This study aimed to examine the efficacy of tadalafil (TAD) and dexlansoprazole (DLP) in treating stomach ulcers caused by dexamethasone (DEX) in male albino Wister rats. Thirty male albino Wister rats were divided into 5 groups (6 rats each): control group received normal saline, positive control group received DEX 5 mg/kg/day intraperitoneal (i.p.) for 7 days, the third group received DLP 30 mg/kg/day orally after DEX, the fourth group received TAD 5 mg/kg/day orally after DEX, and the fifth group received DLP and TAD orally after DEX. Persistence and prevention of ulcers, pepsin activity, mucin content, and histopathological changes were evaluated after each trial. Reduced glutathione (GSH), nitric oxide (NO), and malondialdehyde (MDA) levels were measured in gastric homogenates. Serum levels of prostaglandin E2 (PGE2), tumor necrosis factor-α (TNF-α), and interleukin-10 (IL-10) were also measured. Treatment with either TAD or DLP alone significantly reduced ulcer index (U.I.), pepsin activity, TNF-α, IL-10 and MDA with significant rise in mucin content, PGE2, NO, GSH, and improved the histological alteration compared to DEX group. When TAD and DLP were administered together, there was a more notable decrease in U.I., pepsin activity, gastric MDA, TNF-α, and IL-10 with concomitant more significant increase in mucin content, NO content, and PGE2 production compared to the TAD or DLP groups alone. Compared to each medicine alone, TAD and DLP together have promising therapeutic potential in preventing stomach ulcers caused by DEX.

Introduction

Millions of individuals throughout the world deal with gastric ulcers (GU), a common gastrointestinal disorder that impacts their daily lives in various ways. Worldwide, GU is responsible for 5-10% of deaths, with a prevalence of 20-60 cases per 100,000 people (Ren *et al*., 2020). Free radicals, peroxides, and oxygen ions are examples of reactive oxygen species (ROS) that have been associated to GU. There is an upregulation of lipid peroxidation and pro-inflammatory cytokine release as well as leukocyte recruitment and activation in response to elevated ROS formation in the gastric mucosa (Duran *et al*., 2020).

The stomach epithelial barrier and blood flow are preserved under normal physiological circumstances by the mucosa's defensive mechanisms, which also include chemicals such as prostaglandins, bicarbonate, heat shock protein, growth hormones, and mucus (Lee *et al*., 2017). Harmful chemicals such as gastric acid, bile acid, ethanol, *Helicobacter pylori* (*H. pylori*), and nonsteroidal anti-inflammatory drugs (NSAIDs) can penetrate the gastric mucosa and overwhelm its protective function burden, leading to mucosal damage (Khan *et al*., 2018).

The development of GU can be influenced by a number of different factors, including as unhealthy eating habits, excessive alcohol use, cigarette smoking, stress, and a hereditary susceptibility (Jeon *et al*., 2020). Moreover, frequent use of medicines such as corticosteroids, chemotherapeutic drugs and anticoagulants linked to stomach ulcers (Palle *et al*., 2018). Impairment of the mucosal barrier can lead to intragastric epithelial cell growth, which in turn can break tight junctions and allow the digestive fluids to enter deep tissues. The disease manifests itself initially as a loss of the epithelium layer, and subsequently progresses to deeper tissue damage, erosion, and ulceration (Liu *et al*., 2024).

A powerful corticosteroid, dexamethasone is frequently prescribed to treat a variety of illnesses (Alan and Alan, 2018). Nevertheless, this prescription has a risk of certain adverse effects, such as glaucoma, GU, osteoporosis, immunosuppression, heart problems, and cognitive impairment (Williams, 2018). According to Luo *et al*. (2004), it caused a delay in the healing of rat GU by inhibiting angiogenesis in the stomach. Inhibiting prostaglandin synthetase, which blocks the gastroprotective effect of prostaglandin, and inhibiting peroxidase, which raises endogenous H_2O_2 levels, produces more reactive hydroxyl radicals, and degranulates mast cells, releasing histamine, which may be the cause of increased acid secretion, harm surface epithelial cells causing gastric erosions and increase the risk of gastric mucosa ulceration (Bandyopadhyay *et al*., 1999). Even more harm to the stomach mucosa is caused by a drop in NO levels (McCall *et al*., 1991).

Antacids, proton pump inhibitors (PPIs), histamine H2 receptor blockers (famotidine, ranitidine), and stomach protecting medicines (sucralfate, PGE1 analogues) are among the several anti-ulcerogenic treatments available (Ugan and Un, 2020). PPIs are the most effective as antiulcer drugs (El Mahdy *et al*., 2020). PPIs have consistently demonstrated patient tolerance, excellent protection and generally superior acid suppression capability than earlier medications (Farley *et al*., 2000). One way these drugs work is by inhibiting the H+/K+ ATPase enzyme system, which is responsible for proton pumping in gastric parietal cells. In this mechanism, PPIs attach permanently and covalently to cysteine residues on the proton pump, blocking acid generation until a replacement proton pump regenerates (Sachs *et al*., 2014).

According to Fass *et al*. (2009), one new PPIs that makes advantage of DDR technology is dexlansoprazole modified release (MR) preparation. The drug is supposed to be released twice, first in the proximal small intestine and again in the more distal sections of the small intestine, a few hours apart. Therefore, the strongest inhibitory effect on the proton pump and the longest length of medication retention in the circulation are guaranteed by dexlansoprazole modified release, compared to

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all other PPIs now available (Behm and Peura, 2011; Hershcovici *et al*., 2011). If you have an ulcer in your duodenum or stomach, erosive or reflux esophagitis, or an ulcer caused by NSAIDs, dexlansoprazole can help. The drug is an excellent choice for an antiulcer agent since it has a longer half-life of elimination, meaning its effects last for a longer period of time (Skrzydło-Radomańska and Radwan, 2015).

Tadalafil is an inhibitor of phosphodiesterase-5 (PDE5) that has a lengthy half-life. In order to release cyclic guanosine monophosphate (cGMP), the PDE5 enzyme hydrolyzes it. Therefore, cGMP levels are increased, and NADPH oxidase activity is suppressed as a result of tadalafil-induced PDE5 inhibition. Inhibiting NADPH oxidase activity enhances cellular antioxidant defense mechanisms by decreasing ROS generation and increasing the concentration of antioxidant enzymes, hence minimizing oxidative stress-induced cell damage (Mohamed *et al*., 2022; Sabra *et al*., 2024). PDE5 inhibitors like vardenafil and sildenafil are similar to tadalafil. It is recommended for the treatment of benign prostrate hyperplasia, coronary heart disease, and erectile dysfunction (Ajibo *et al*., 2022a; Hamdy *et al*., 2022).

Tadalafil works by raising cGMP levels, which will have anti-inflammatory benefits and increase in blood flow to gastrointestinal tissues (Ahmed Amar *et al*., 2019). Endogenous nitric oxide synthase is enhanced. It is well-known that NO is a vasodilator due to its ability to improve blood flow in gastrointestinal tissues and minimise tissue deterioration. As a result, tadalafil can lessen the severity of ulcers (Ajibo *et al*., 2022b).

This work tested and compared the anti-inflammatory, antioxidant and anti-apoptotic effects of PPIs with PDE5 inhibitors, a recently recommended therapy medication using a rat model of dexamethasone-induced GU. Therefore, the current study sought to assess the therapeutic effects of tadalafil and dexlansoprazole in rats with GU produced by dexamethasone and explored potential causes.

Materials and methods

Animals

A total of 30 adult male albino Wistar rats weighing 170 to 210 g were purchased from the animal house at Assiut University's Faculty of Veterinary Medicine in Egypt. Rats were housed in standard specific pathogen free facilities, maintained at 24 ± 2ºC; 60–70% relative humidity and12 h/12 h light/dark cycle and acclimated for 1 week before start of experiment. Food and water were available ad libitum. All the animal experiments were conducted in accordance with the guide for the care and use of animals of the National Institutes of Health (NIH, 1985) and approved by the local ethical and scientific committee of Faculty of Medicine - Assiut University with approval number (17300966, 13/12/2022).

Drugs and chemicals

Dexamethasone (8 mg/2 ml) was purchased from Amriya company in the form of liquid ampoules, which was administered intraperitoneal (i.p.). Tadalafil (TAD) and Dexlansoprazole (DLP) were graciously provided by Egyptian International Pharmaceutical Industries Company (EIPICO) in the form of powder, that were given orally. Tadalafil was dissolved in saline. But, dexlansoprazole was dissolved in distilled water.

Experimental design

The animals were divided into five groups of six rats each:
Group I (control group); The rats in this group were given 0.2 ml of normal saline orally every day for seven days.
Group II (DEX group); In which gastric ulceration was induced by i.p. injection of DEX 5 mg/kg/day for 7 days (Swamy *et al*., 2011).
Group III (DEX+DLP group); In which animals treated with DEX (5 mg/kg/day) and DLP in a dose of 30 mg/kg/day orally for 7 days (Mohamed and Kamel, 2023).
Group IV (DEX+ TAD group); In which animals were treated with DEX (5 mg/kg/day) and TAD in a dose of 5 mg/kg/day orally for 7 days (Kolawole and Francis, 2012).
Group V (DEX+DLP+ TAD); in which the animals concurrently treated with DEX (5 mg/kg/day), DLP 30 mg/kg/day and TAD 5 mg/kg/day orally for 7 days.

Induction of gastric ulcer

Dexamethasone (DEX) in a dose of 5 mg/kg/day was injected intraperitoneally for 7 days to induce gastric ulcers (Rizk *et al*., 2017).

Animal handling and samples preparation

After the seventh day, rats were anesthetized with 4% isoflurane, which obtained from Kahira Pharmaceuticals and Chemical Industries Co. (Cairo, Egypt) (Takeuchi *et al*., 2014). Blood samples were taken from the heart (Ahmed *et al*., 2020). Blood was centrifuged at 3000 rpm for 10 minutes to remove serum (Mohamed and Kamel, 2023). The serum was subsequently maintained at -80°C until analysis and used to analyze inflammatory markers (Wang *et al*., 2017). Animals were sacrificed via cervical dislocation. The excised stomach was opened along the larger curvature, its content was collected in a tube and then thoroughly cleaned with cold saline for 30 minutes. Stomach wall was classified into specimens and used for measuring oxidative stress parameters, for histopathological and immunohistochemical examinations and for scanning electron microscopy.

Tissue homogenate preparation

According to Mohamed and Kamel (2023), a part from the stomach from each sample was weighed individually and homogenized in ice-cold potassium phosphate buffer (pH 7.4). The homogenates underwent a 10-minute, 3500 rpm centrifugation at 4°C. The recovered supernatant was stored at -80°C to facilitate the measurement of oxidative stress. For histological examination, additional glandular stomach specimens were preserved in 10% neutral buffered formalin (Liu *et al*., 2021).

Analysis of gastric juice

The greater curvature of each stomach was opened and the contents were poured into a centrifuge tube. To eliminate any solid debris, the tube was spun for 10 minutes at 3000 rpm (Lotfy *et al*., 2022). The volume of supernatant was then collected for pepsin and mucin analysis (El-Saka *et al*., 2014).

Estimation of gastric pepsin activity

Gastric juice was used to measure pepsin in accordance with Boushra *et al*. (2019) description. Using hemoglobin as a foundation. The pepsin content was represented in µM of tyrosine liberated/ml.

Estimation of gastric mucin content

According to Corne's (1974) description, the amount of gastric mucus in gastric juice was measured. The findings were given as mg% hexose.

Determination of serum levels of inflammatory markers

Blood collected from each animal was centrifuged at 3000 rpm for 10 min and the volume of the supernatant was measured. PGE2 and TNF-α were measured by enzyme linked immunosorbent assay (ELISA) Kits (Elabscience biotechnology Co., Ltd, Wuhan, China) according to the

method described by manufacturers (Zhou *et al*., 2020). IL-10 was assessed by ELISA kits (Thermo Fisher Scientific, MA, USA) according to the manufacturer's instructions (Duran *et al*., 2020).

Determination of oxidative stress and antioxidant parameters

According to a previous study (Abdel-Tawab *et al*., 2020; Sabra *et al*., 2023), malondialdehyde (MDA), nitric oxide (NO), and reduced glutathione (GSH) were assessed spectrophotometrically in tissue homogenates using colorimetric kits from Bio-Diagnostic (Cairo, Egypt).

Gastric ulcer index and scoring

The stomach was carefully opened along its larger curvature to determine the ulcer index (U.I.). Flattened stomach samples were then examined and photographed. An arbitrary scale was used to grade the U.I. scores (Singh *et al*., 2008).

Protective index (P.I.)= (UI model group-UI treated group)/(UI model group)X 100%

Evaluation of ulcer index was described as follow (0 = no injury, 0.5–1= hyperaemia, 1–2 = hemorrhagic lesions, 2–3 = one to five minor ulcers, 3–4 = numerous minor ulcers, 4–5 = 1-3 big and 1-2 little ulcers, 5–6 = numerous large and tiny ulcers, 6 = stomach with several holes or ulcers).

Histopathological examination

Neutral buffered formalin 10% was used to fix tissue samples from the stomach. Next comes dehydration using increasing alcohol grades, xylene clearing and paraffin embedding. Tissue sectioning that is 4–5 microns thick, followed by staining with hematoxylin and eosin (H&E) (Banchroft *et al*., 1996).

Scanning electron microscopy (SEM)

Immediately after, representative stomach specimens were cleaned with regular saline. Afterwards, the samples were preserved for 24 hours at 4°C in a solution of 2.5% paraformaldehyde and 5% glutaraldehyde in 0.1 M sodium phosphate buffer. After that, wash the area with 0.1 M sodium phosphate buffer (pH 7.3). Next, dehydrate the area using an ascending series of ethanol concentrations: 30, 50, 70, 90, 100% for two days, and finally, amyl acetate for two days. Liquid carbon dioxide was used to apply critical point drying to the samples. Each sample was painted silver and adhered to metallic blocks. A 15 nm layer of uniform gold coating was applied to the samples using a gold sputter coating device. The samples were imaged in the electron microscopy unit at Assiut University in Egypt and analyzed using a JEOL (JSM 5400 LV) scanning electron microscope operating at 15–25 kV (Bozzola and Russell, 1999).

Immunohistochemistry for nuclear factor kappa B (NF-kB) detection

Using immunohistochemical staining, NF-kB was examined. The method was carried out in accordance with Khalil *et al*. (2020). Sections were dewaxed and immersed in a solution of 0.05 M citrate buffer, pH 6.8 for antigen retrieval. These sections were then treated with 0.3 % H_2O_2 and protein block. Then, sections were incubated with polyclonal anti-NF-κB P65 (Santa cruz, Cat# (F-6): sc-8008, 1:100 dilution). After rinsing with phosphate buffered saline, they were incubated with a goat anti-rabbit secondary antibody (Cat# K4003, EnVision+™ System Horseradish Peroxidase Labelled Polymer; Dako) for 30 min at room temperature. Slides were seen using a 3,3'-diaminobenzidine (DAB) kit, and Mayer's hematoxylin was used as a counterstain after that. For each of the eight high power fields (HPF), the staining intensity was evaluated and shown as a percentage of positive expression in a total of 1000 cells.

Statistical analysis

Data were represented as mean ± SE of 6 observations. The significance of differences between groups was analyzed using One-way analysis of variance (ANOVA) followed by Bonferroni for multiple comparisons as appropriate. Differences were considered significant at $P < 0.05$. The data and statistical analysis comply with the recommendations on experimental design and analysis in pharmacology. All statistical analyses were calculated with Prism software (Graph-Pad Software Inc, version 9.0.0).

Results

Effects of the tested drugs on pepsin and mucin level in gastric juice

Treatment with dexamethasone 5 mg/kg i.p. daily for 7 days caused a significant increase in pepsin level with significant decrease in mucin content compared with negative control group at $p < 0.0001$. Treatment by dexlansoprazole, tadalafil and combination of DLP+ TAD in rats with GU induced by DEX demonstrated a significant decrease in pepsin level with a significant increase in mucin content compared with DEX group at $p < 0.0001$. But, the decrease in pepsin and increase in mucin was less than the effect of combination of DLP+TAD. No significant differences were found between the Tadalafil- treated group and dexlansoprazole- treated group in pepsin and mucin level (Table 2).

Table 1. Ulcer index and protective index of tadalafil, dexlansoprazole and combination of DLP+TAD in rats with GU induced by DEX.

Animal group	Ulcer index	Protective index
Control	Nill	
DEX induced	5 (0.4)	-
DEX + DLP	3 (0.34) δ	38.29%
DEX + TAD	2 (0.1) δ	53.19%
DEX+DLP+TAD	2.3 (0.12) δ	63.82%

Ulcer index values are expressed as mean (S.E.M). $^{\delta}P < 0.05$ vs. Dex group

Effects of the tested drugs on the level of inflammatory markers (PGE2, TNF-α, IL-10) in serum

There was a significant increase in serum TNF-α and IL-10 and a significant decrease in serum PGE2 in the DEX group compared with the negative control group at $p < 0.0001$. In contrast, dexlansoprazole (30 mg/kg), tadalafil (5 mg/kg) and combination of DLP+ TAD in rats with GU showed a significant increase in PGE2 level with significant decrease in serum TNF-α and IL-10 at $p < 0.0001$ compared with DEX group. But, combination of DLP+TAD caused a more positive result than each drug alone. Tadalafil-treated group showed no significant differences in serum

Table 2. Effect of tadalafil (5 mg/kg) and dexlansoprazole (30 mg/kg) alone, and their combination on pepsin and mucin against dexamethasone induced gastric ulcer in rats.

Groups	Control	DEX	DEX+DLP	DEX+TAD	DEX+DLP+TAD
Pepsin (μmol/ml)	263.3±7.279^{a}	524.8±2.414^{b}	432.8±7.743^{c}	422.3±8.917^{c}	331.8±5.730^{d}
Mucin (mg % hexose)	492.8±3.582^{a}	228.8±5.128^{b}	323.5±4.965^{c}	337.5±7.986^{c}	437.2±4.799^{d}

Data represent mean ± SE of six observations. DEX: dexamethasone, DLP: dexlansoprazole, TAD: tadalafil. Values with different superscript letters are significantly different ($P<0.0001$), a: compared to DEX-group, b: compared to control-group, c: compared to DLP+TAD-group, d: compared to DLP-group

PGE2, TNF-α and IL-10 compared with dexlansoprazole- treated group (Table 3).

Effects of the tested agents on the level of oxidative stress markers (MDA, NO, GSH) in gastric homogenate

The results showed that dexamethasone caused a significant increase in gastric MDA with significant decrease in gastric NO and GSH compared with negative control group at $p < 0.0001$. The observed changes in these parameters were significantly attenuated by administration of DLP, TAD and combination of DLP+TAD in rats with GU at $p < 0.0001$. But, Co-administration of DLP and TAD produced higher effect as compared to DLP or TAD alone. Tadalafil-treated group showed no significant differences in gastric MDA and GSH with significant decrease in gastric NO compared with dexlansoprazole- treated group at $p < 0.01$ (Table 4).

Histopathological examination

Histopathological examination of control group revealed normal structure of the gastric epithelium (Fig. 1A). DEX- induced group revealed presence of multiple ulcers in the form of small and large ulcers. Necrosis of the gastric mucosa with infiltration of inflammatory cells as neutrophils as well as congestion and hemorrhage were noticed (Figs. 1B-F). Examination of dexlansoprazole- treated group showed presence of small ulcers and focal accumulation of mononuclear cells in lamina propria (Figs. 2A, B). Tadalafil- treated group showed minute small ulcers and presence of granulation tissues with congestion of blood vessels in submucosa (Figs. 2C, D). While examination of combination of DLP+TAD group revealed normal appearance of the gastric mucosa (Fig. 2E).

Immunohistochemical results and statistical analysis

Immunohistochemical analysis of gastric tissues of the negative control group showed mild the immunostaining of NFκB-P65 antibody (1.833 ± 0.4773) (Fig. 3A). DEX-induced group showed statistically significant increase in the immunostaining of NFκB-P65 antibody (23.67± 3.658) compared with negative control group (Fig. 3B). Examination of the group that treated with dexlansoprazole revealed statistically significant decrease in the immunostaining of NFκB-P65 antibody (12.00 ±1.291) compared with DEX-induced group (Fig. 3C). Examination of the group that treated with tadalafil revealed statistically significant decrease in the immunostaining of NFκB-P65 antibody (15.50 ±1.544) compared with DEX-induced group (Fig. 3D). Examination of the group that treated with combination of DLP+TAD revealed statistically significant decrease in the immunostaining of NFκB- P65 antibody (3.167 ±0.4773) compared with DEX-induced group (Fig. 3E).

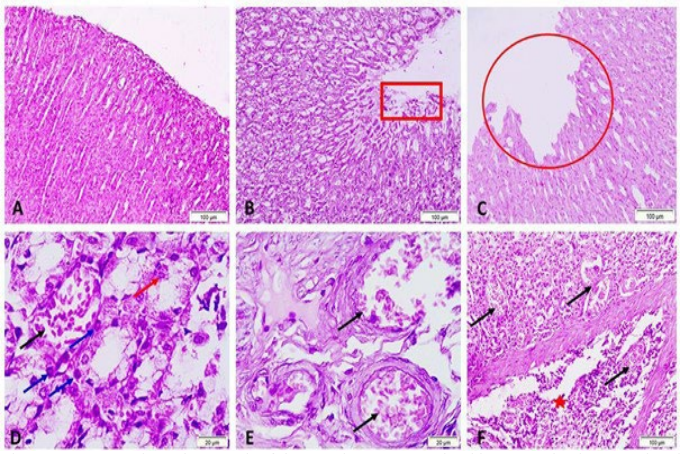

Fig. 1. Representative micrograph of the gastric mucosa stained by HE stains in control and induction group. A) Control group showing normal appearance of the gastric mucosa. B-F) DEX- induced group showing multiple ulcers; small (red rectangle) and large ulcer (red circle), congestion of the blood vessels (black arrows), necrosis of the gastric epithelium (red arrow), infiltration of neutrophils (blue arrows) and hemorrhage (star).

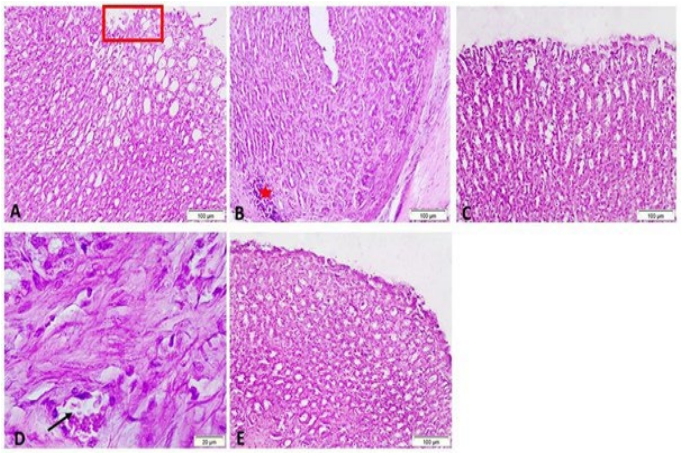

Fig. 2. Representative micrograph of the gastric mucosa stained by HE stains in the treated groups. A, B) Dexlansoprazole- treated group showing small ulcer (rectangle) and focal accumulation of mononuclear cells in lamina propria (star). C, D) Tadalafil- treated group showing minute small ulcers and granulation tissue with congestion in submucosa (arrow). E) Combination of DLP+TAD group showing normal appearance of the gastric mucosa.

Table 3. Effect of tadalafil (5 mg/kg) and dexlansoprazole (30 mg/kg) alone, and their combination on inflammatory markers (PGE2, TNF-α, IL-10) against dexamethasone induced gastric ulcer in rats.

Groups	Control	DEX	DEX + DLP	DEX + TAD	DEX+DLP+TAD
PGE_2 (pg/ml)	48.06±1.487[a]	15.75±0.9014[b]	27.34±0.9623[c]	29.26±0.5959[c]	41.00±0.8946[d]
TNF-α (pg/ml)	79.28±3.381[a]	107.9±4.176[b]	83.06±1.575[a]	68.86±1.629[a]	78.71±1.756[a]
IL-10 (pg/ml)	63.79±0.9482[ac]	91.71±3.168[b]	63.56±2.233[a]	63.56±2.233[a]	59.04±0.7607[c]

Data represent mean ± SE of six observations. DEX: dexamethasone, DLP: dexlansoprazole, TAD: tadalafil, PGE2: prostaglandin E2, TNF-α: tumor necrosis factor- alpha, IL-10: interleukin -10. Values with different superscript letters are significantly different ($P<0.0001$), a: compared to DEX-group, b: compared to control-group, c: compared to DLP+TAD-group, d: compared to DLP-group

Table 4. Effect of tadalafil (5 mg/kg) and dexlansoprazole (30 mg/kg) alone, and their combination on oxidative stress markers (MDA, NO, GSH) against dexamethasone induced gastric ulcer in rats.

Croups	Control	DEX	DEX+DLP	DEX +TAD	DEX+DLP+TAD
MDA (nmol/g)	3.950±0.4537[a]	19.40±0.6237[b]	11.30±0.7776[c]	11.33±0.7712[c]	7.214±0.7587[d]
NO (µmol/l)	375.0 ± 4.550[a]	60.66 ±3.179[b]	283.4±11.50[c]	243.0 ±3.796[d]	351.1±10.88[a]
GSH (mg/g)	48.55 ± 1.051[a]	31.35 ± 1.440[b]	43.02±0.7920[c]	42.28±0.8362[c]	50.67 ± 1.190[a]

Data represent mean ± SE of six observations. DEX: dexamethasone, DLP: dexlansoprazole, TAD: tadalafil, MDA: malondialdehyde, NO: nitric oxide, GSH: reduced glutathione. Values with different superscript letters are significantly different ($P<0.0001$), a: compared to DEX-group, b: compared to control-group, c: compared to DLP+TAD-group, d: compared to DLP-group

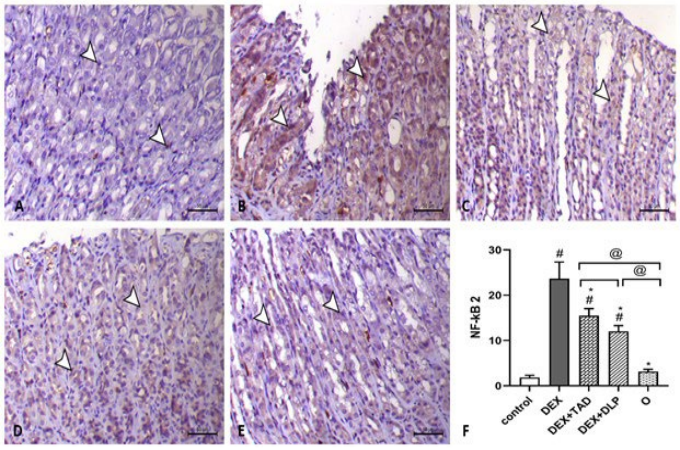

Fig. 3. Immunohistochemistry photomicrograph of NFκB-P65 IHC of the gastric mucosa of the studied groups. A) Control group showing mild expression of NFκB-P65 antibody within the few epithelial cells of the gastric glands (arrowheads). B) DEX-induced group showing marked increase of both cytoplasmic and nuclear expression of NFκB-P65 antibody within the gastric glands around the ulcer (arrowheads). C) Dexlansoprazole- treated group showing decrease the cytoplasmic and nuclear expression of NFκB-P65 antibody within the gastric glands (arrowheads). D) Tadalafil- treated group showing decrease the immunostaining of NFκB-P65 antibody within the lining epithelium of the gastric glands (arrowheads). Combination of DLP+TAD group showing marked decrease the expression of NFκB-P65 antibody which appears only within the cytoplasm of within the gastric glands (arrowheads), X200, bar = 50 µm. F). Values are expressed as mean ± SE (n=6). # Significant difference at $p < 0.0001$ compared with negative control group, * Significant difference at $p < 0.0001$ compared with DEX group and @ Significant difference at $p < 0.001$ compared with other different treatment.

Scanning electron microscopy

Scanning electron microscopical examination of the stomach in control group showed normal appearance of the gastric epithelium (Fig. 4A). Examination of induction group revealed presence of multiple large ulcers with destruction of epithelium of the gastric mucosa (Figs. 4B, C). While in the combination of DLP+TAD group showed improvement of the gastric epithelium which revealed normal appearance of the gastric mucosa (Fig. 4D).

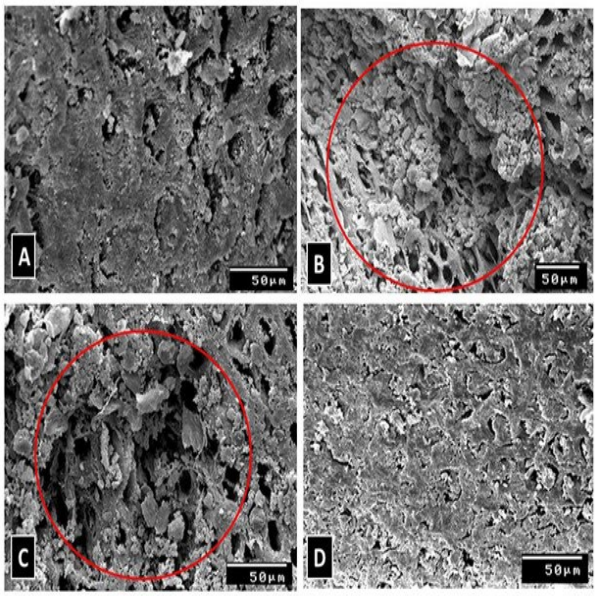

Fig. 4. Scanning electron micrograph of gastric mucosa in control group with normal epithelium (A), Scanning electron micrograph of induction group showing large ulcers in the gastric mucosa (red circles) (B, C), Combination of DLP+TAD group showing normal appearance of the gastric mucosa (D).

Discussion

Gastric ulcers are a common health problem that affects around 5% of the population (Boligon *et al*., 2014). Damage to the stomach's inner surface from hydrochloric acid, *H. pylori*, and free radicals occurs when the body's defense mechanisms are overwhelmed (Cheng *et al*., 2013). Ulcers are associated with high rates of mortality and morbidity because of the many complications they can cause, such as bleeding, perforation, penetration, and blockage of the stomach outlet (Milosavljevic *et al*., 2011; Abdel-Tawab *et al*., 2020).

Dexamethasone and other corticosteroids have been used to treat a range of diseases for almost half a century because of its anti-inflammatory and immunosuppressive effects. Regardless, gastric ulcers have been associated with long-term corticosteroid use. Also, corticosteroids can cause other side effects in the body such as glaucoma, osteoporosis, Immunosuppression, cardiac problems and cognitive impairment (Luo *et al*., 2003; Rateb *et al*., 2021).

In our results, dexamethasone was injected daily for 7 days (5 mg/kg i.p.) caused gastric ulceration and gastritis. In agreement with the study of Thippeswamy *et al*. (2010); Swamy *et al*. (2011); El Zahaby *et al*. (2017); Rizk *et al*. (2017); Marian *et al*. (2019) and Rateb *et al*. (2021) who reported that DEX treatment led to GU in rats by different doses. DEX damaging the surface epithelial cells and increasing the risk of gastric ulcers by reducing the generation of prostaglandins (PGs), which are necessary for the formation of the mucous barrier layer. Additionally, disrupt the production of gastric mucus, the secretion of gastric bicarbonate, which weakens the gastric mucosa, lowers NO levels, increases the amount of acid produced by the stomach and increases pepsin, enhances gastrin, and parietal cell hyperplasia with increased acid secretion, all of which lead to gastric mucosa lesions and ulcers (Luo *et al*., 2003, Rizk *et al*., 2017, Rateb *et al*., 2021).

Propepsin, the precursor of pepsin, is activated by hydrochloric acid and has the ability to break down protein molecules (Liu *et al*., 2024). The stomach produces an adherent layer of mucus lining high in bicarbonate to protect itself from the digesting actions of pepsin. Mucin molecules are used to make mucus. Glycoproteins called mucins serve as a filter to stop infections and other dangerous chemicals from moving freely. Damage to the mucin layer and mucus production leaves the body vulnerable to infections like *H. pylori* (Ajibo *et al*., 2022b).

In the current investigation, we noticed that, in comparison to the negative control group, pepsin was highly enhanced and mucin was dramatically decreased in DEX-induced GU. These results were in line with studies by Bandyopadhyay *et al*. (1999) and Luo *et al*. (2003) that showed rats treated with DEX had significantly higher levels of pepsin and lower levels of mucin. Similar results were also reported by Thippeswamy *et al*. (2010) who studied Pylorus Ligation Plus dexamethasone induced ulcer model in rats in response to oral injection of dexamethasone at a dose of 5 mg/kg daily in Wistar rats. A statistically significant increase in pepsin level with a decrease in mucin content in rats compared to the negative control rats was reported...

One of the key mechanisms for stomach mucosal damage is inflammation (Zhao *et al*., 2013). Damage caused by oxidative stress in the stomach can encourage neutrophils to aggregate and infiltrate the stomach mucosa, as well as control the transcription and synthesis of various pro- inflammatory cytokines like TNF-α and anti-inflammatory factors like IL-10 (Byeon *et al*., 2018).

Tumor necrosis factor-alpha is a pro-inflammatory mediator that stimulates tissue damage through adhesion molecule increase, resulting in leukocyte recruitment. Neutrophils accumulating can cause more tissue damage by producing more ROS, which can harm stomach tissue (Musumba *et al*., 2009) and disruption of the stomach's microcirculation as well as the development of a stomach mucosal ulcer (Mansouri *et al*., 2015). Elevated TNF-α levels can trigger the release of cytokines such as IL-6 and IL-1β, leading to an inflammatory response (Wang *et al*., 2018).

IL-2, IL-1β, IL-6, and IL-18 are the key interleukins (ILs) studied in the etiology of GU. Intercellular communication, which includes cell migration, proliferation, maturation, and adhesion and is essential for the inflammatory response, is the primary function of ILs in the immune system. Interleukins are involved in both chronic and acute inflammation (Liu *et al*., 2021). An important cytokine that lowers inflammation, inhibits the immune system, and has the ability to halt TNF-α production is IL-10 (Antonisamy *et al*., 2016).

Prostaglandin E2 is a key mediator in maintaining the integrity of the stomach mucosal defense and in the healing of GU. PGE2 plays a key role in preventing and treating ulcers brought on by noxious compounds by controlling gastric acid secretion, the release of cytotoxic substances, stabilizing mast cell membranes and stimulating the healing process of tissue. In addition to causing GU, the lower level of PGE2 in the stomach mucosa exacerbates pre-existing GU (Zhou *et al*., 2020).

In this study, a significant elevation was seen in the serum TNF-α and IL-10 with significant decline in the serum PGE2 level in DEX –induced GU rats compared with negative control group. DEX caused significant elevation in TNF-α and significant decline in PGE2 and this was in harmony with Chi (2009) and Thippeswamy *et al*. (2010) who reported that dexamethasone plus pylorus ligation led to a significant elevation in TNF-α and significant decline in PGE2. The results were in disagreement with Abdel-Tawab *et al*. (2020) who reported that indomethacin treatment led to a significant decline in IL-10. Conversely, Qin and Qiu (2019) demon-

strated that dexamethasone can balance anti-inflammatory and inflammatory responses by suppressing TNF-α and increasing IL-10 expression in serum, reducing lung tissue injury.

Furthermore, Swarnakar *et al*. (2005) discovered that ulcerogenesis appeared to be primarily caused by DEX-induced stomach TNF-α content elevation at the ulcer site. DEX likely inhibits PGs synthesis, which inhibits TNF-α release from macrophages, leading to an increase in TNF-α levels. (Kunkel *et al*., 1986). DEX may up-regulate stomach TNF-α, leading to a decrease in mucosal NO. This action is consistent with the results of Bauer *et al*. (1997), who recorded that TNF-α inhibits constitutive NO and provides stomach protection via regulating cytokine production.

Numerous pieces of data demonstrate how oxidative stress plays a role in the pathogenesis of GU. Oxidative stress in the stomach mucosa results from an imbalance between ROS and antioxidants, which causes GU (Danışman *et al*., 2023). Dexamethasone is linked to the pathophysiology of GU by inducing oxidative stress by lipid peroxidation and depleting antioxidants in the mucosa of the stomach (Thippeswamy *et al*., 2010) and preventing the action of prostaglandin syntheses and peroxidase, two crucial gastroprotective enzymes (Swamy *et al*., 2011).

Due to the body's metabolism of DEX, which results in the production of superoxide anions and hydroperoxyl free radicals, dexamethasone has been shown to cause ROS to overrun (Rizk *et al*., 2017; Rateb *et al*., 2021). Furthermore, there is a direct correlation between the activation and accumulation of neutrophils in the damaged stomach mucosa and the excessive generation of free radicals by dexamethasone. Tissue damage is caused by these invading neutrophils' excessive production of superoxide radical anion (Marian *et al*., 2019).

Increased MDA levels indicate lipid peroxidation, which is caused by free radicals directly attacking proteins, nucleic acids, and lipid content of the gastric mucosal membrane (Antonisamy *et al*., 2016). MDA, which is produced when polyunsaturated fatty acids in cell membranes peroxide, is frequently employed as a trustworthy signal when tissue lipid peroxidation is occurring (Abdeen *et al*., 2019).

One of the most significant and powerful antioxidants is reduced glutathione (GSH). Because of its sulfhydryl groups which scavenge ROS and consequently stop lipid peroxidation, it shields cells from oxidative stress. In addition, GSH can neutralize H_2O_2 and function as a cofactor for the enzyme glutathione peroxidase (GPx). It has been demonstrated that dexamethasone causes the amount of GSH in stomach tissue to decrease. This decrease causes ROS accumulation to rise, which in turn causes lipid peroxidation to rise (Beiranvand and Bahramikia, 2020).

A natural defense mechanism of gastric cells, NO provides gastroprotective qualities against several hostile chemicals (Samini *et al*., 2002). By regulating gastric motility, microcirculation, and the production of mucus and alkaline, it helps to maintain mucosal integrity (Tsukimi and Okabe, 2001). El Mahdy *et al*. (2020) and Sánchez-Mendoza *et al*. (2020) stated that NO regulates blood flow, mucus production, acid levels, and membrane lipid peroxidation in stomach tissues.

The enzyme known as nitric oxide synthase (NOS) is in charge of producing nitric oxide. The two main types of NOS are inducible NOS and endothelial NOS (eNOS) (iNOS). Numerous studies have shown that NO, which is produced by iNOS, has a role in cellular pathogenesis. Tissues are protected from many pathogenic circumstances by NO, which is generated by eNOS. There is evidence that patients suffering from peptic ulcers exhibit higher levels of iNOS expression (Bhattacharyya *et al*., 2014).

The results of this study demonstrated an increase in the level of MDA and reduce GSH in stomach homogenate in DEX-treated group in comparison with negative control group and these results were similar to that reported by Rateb *et al*. (2021) who investigate the effect of vitamin D on dexamethasone induced metabolic disturbance and gastric ulcer. Daily intraperitoneally dexamethasone (1 mg/kg) for two weeks caused significant increase in MDA and reduce GSH level. In addition, the results demonstrated by Manjari and Das (2000) corroborated by our own findings that DEX at 5 mg/kg/day for 10 days reduced NO levels in the stomachs of rats were in agreement with our findings.

The aforementioned modifications align with the findings of our study, which demonstrated that the i.p. administration of DEX resulted in severe hemorrhagic ulcerative lesions, as indicated by a noteworthy rise in U.I. (U.I.=6) and lower P.I. (P.I.=0%) levels. This was verified by a histological investigation, which revealed that the stomach epithelium in this experiment had numerous ulcers, vacuolated surface epithelial cells, infiltration of mononuclear cells, hemorrhagic regions, mucosal necrosis and congested capillaries. Similar findings from a recent study have been published by Rizk *et al*. (2017); Marian *et al*. (2019) and Rateb *et al*. (2021) who investigated the effect of Montelukast and Nigella Sativa Oil on dexamethasone induced metabolic disturbance and gastric ulcer. Daily orally dexamethasone (5 mg/kg) for 7 days caused rise in U.I. and lower P.I. levels with vacuolated surface epithelial cells with mononuclear cells infiltration and hemorrhagic areas.

One of the ways tadalafil prevents ulcers is by acting as a NO donor, which is known to have anti-secretory and mucogenic properties. This quality causes the stomach mucosa and other non-erectile tissues to experience an increase in blood flow (Kolawole and Francis, 2019). Additionally, tadalafil has anti-inflammatory, anti-oxidative, and anti-apoptotic characteristics (Laxmi *et al*., 2019). Theoretically, this establishes tadalafil's use in GU therapy. Concerning this matter, the purpose of this research was to examine the possible curative impact of tadalafil on DEX-induced GU in rats in comparison to the conventional medication dexlansoprazole.

Proton pump inhibitors such as dexlansoprazole are used to treat and sustain the healing of erosive and reflux esophagitis, ulcers caused by NSAIDs, and duodenal or stomach ulcers. PPIs have been recommended as gastroprotective medicines in more recent treatment plans because of their potent anti-inflammatory and antioxidant properties in addition to their capacity to inhibit the generation of acid. PPIs can reduce ROS production, which improves their capacity to combat inflammation and function as antioxidants (Mohamed and Kamel, 2023).

when compared to the DEX-treated group, the usage of the tested medications significantly elevated the amount of mucin and dramatically lowered the level of pepsin in the current investigation. In agreement with the study of Thippeswamy *et al*. (2010) who investigated the PPIs significantly decreased in pepsin level and significantly increased in mucin level in dexamethasone plus pylorus ligation induced GU in male rats. PDE5 inhibitors demonstrated a noticeably higher mucin deposition than the DEX-treated group. This was consistent with the analysis of Maziero Alves *et al*. (2021).

Furthermore, the results of this study revealed that oral administration of tadalafil, dexlansoprazole and combination in rats with GU induced by DEX caused a reduction in TNF-α and IL-10 with increase in PGE2 compared with DEX-treated group. In agreement with Mohamed *et al*. (2022) who reported that tadalafil and dexlansoprazole significantly decreased in TNF-α with significant increase in PGE2. Conversely, Matloub and Manna (2010) reported that PDE5 inhibitors administration increased anti-inflammatory cytokines (IL-10) (Matloub and Manna, 2010).

On the other hand, the oxidative stress caused by DEX was considerably reduced by tadalafil, dexlansoprazole, and their combination in rats treated with DEX. Stomach GSH and NO levels increased while gastric MDA levels decreased significantly when compared to the ulcer group. These results corroborated those of earlier research showing that tadalafil significantly reduces stomach MDA while raising GSH and NO, an antioxidant mechanism via which it exerts its anti-ulcer effects (Medeiros *et al*., 2008; El Mahdy *et al*., 2020; Maziero Alves *et al*., 2021; Mohamed *et al*., 2022). In contrast with, as compared to the DEX-treated group, researchers found that NO levels were significantly lower in the groups given tadalafil and PPIs. This was because tadalafil reduced iNOS expression while increasing eNOS expression (Azouz *et al*., 2020; Mohamed *et al*., 2022).

The rats in the group that received tadalafil, dexlansoprazole, and their combination in rats with GU showed no signs of ulceration and had significantly lower U.I. (3-4, 1-2 and 0.5-1; respectively) and higher P.I. (53.19, 28.29 and 63.82 %; respectively) levels compared to the rats in DEX group. Furthermore, histological data corroborated these findings, showing that these medicines successfully repaired the stomach histopathology abnormalities caused by DEX. Curiously, compared to tadalafil or dexlansoprazole alone, combination medicines had a more robust healing effect. These results agreed with those of earlier research (Mohamed *et al*., 2022).

We investigated molecular NF-kB expression in gastric mucosa to see if innate immunity was associated with gastric ulcer therapy or prevention. Treatment with DEX elevated NF-kB expressions in gastric mucosa. The expressions returned to normal levels after treatment with tadalafil and dexlansoprazole. In order to increase gut immunity, decrease GU formation, and minimize tissue damage, these data indicated that tadalafil's regulation of the NF-kB signaling pathway is critical.

Nuclear factor kappa B is a transcription factor that controls the expression of genes linked to inflammation and cell damage (Kamphan *et al*., 2017). When healthy cells are at rest, NF-kB is coupled to inhibitor-kB (I-kB) in the cytosol where it is inactive. The phosphorylation of I-kB and its dissociation from NF-kB which results in its translocation into the nucleus, are made possible by inflammation and inflammatory stimuli. Only in ulcerated tissue may activated NF-kB be found and this state persists until stomach ulcers (Takahashi *et al*., 2001).

Additionally, NF-kB activation causes neutrophil infiltration by up-regulating adhesion molecule expression, including intercellular adhesion molecule-1 (ICAM-1) (An *et al*., 2020). ICAM-1 promotes neutrophil adhesion and leukocyte transendothelial migration which worsens mucosal tissue injury (Konturek *et al*., 2000). ROS stimulate NF-kB translocation which is essential for matrix metalloproteinase (MMP) production and activity during ulceration in the submucosal zone of stomach tissues. The primary transcription factor for inflammation, NF-kB controls the ex-

pression of growth factors and pro-inflammatory cytokines. The overexpression of pro-inflammatory mediators like TNF- α and IL-6 is further encouraged by the overexpression of NF-kB subunits (P65 and P50) and their translocation to the nucleus (Chakraborty *et al*., 2012).

When comparing the ulcer group to the negative control group, this study found that NFκB-P65 antibody expression in the gastric glands around the ulcer was significantly higher in the ulcer group, both in the cytoplasm and the nucleus. These results were consistent with those of earlier research (Ahmed *et al*., 2020; Duran *et al*., 2020; Yoo *et al*., 2020; Kadasah *et al*., 2021). In contrast to the DEX-treated group, the expression of the NFκB-P65 antibody within the stomach glands was significantly reduced in the group treated with tadalafil, dexlansoprazole, or both. The results of this investigation corroborate those of Duran *et al*. (2020), who found that PPIs considerably reduced NF-κB expression. Despite several research demonstrating that medications can alter NF-κB expression in different tissues, none studies have yet examined tadalafil's impact on NF-κB expression in GU.

Scanning electron microscopy (SEM) has recently become widely used for the investigation of various biological specimens. In this way, SEM may be utilized to study the genesis and progression of stomach ulcers, determine the constituents of the epithelium that has regenerated, and monitor pre-eruptive changes in the cells (Aruin and Chikunova, 1980).

The gastric epithelium in the control group seemed normal when examined using scanning electron microscopy in our investigation. Further analysis of the ulcer group to the negative control group showed that the ulcer group had more severe stomach mucosal epithelial damage and numerous big ulcers. The stomach epithelium improved in the combination-drug group, indicating normal gastric mucosa appearance, in contrast to the DEX group. These results agreed with those of earlier research by El Beshbishy *et al*. (2010); Chakraborty *et al*. (2012) and Zaki and Mohamed (2014) who reported that dexamethasone (1 mg/kg) i.p. showed gastric ulcer extending up to the muscularis mucosa with abnormal surface epithelial cells within the gastric ulcer area.

Conclusion

The results of this trial demonstrated that tadalafil significantly decreased the occurrence of GU caused by dexamethasone injections. In addition, it provides evidence that tadalafil's therapeutic efficacy is caused by its antioxidant qualities, decrease of inflammation and mitigation of oxidative stress, as well as its lowering of the NF-κB signaling pathway. This means that tadalafil can prevent the formation of ulcers. The effects are quite similar to the reference medicine, dexlansoprazole. The combination of tadalafil and dexlansoprazole are more effective in modulating the biochemical and histological markers generated by dexamethasone than either drug alone. The necessity to investigate many other signaling pathways for their possible involvement in tadalafil's therapeutic impact may be considered in future studies.

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Conflict of interest

The authors declare that they have no conflict of interest.

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