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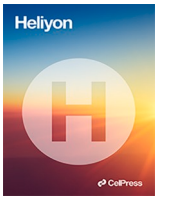

Research article

Troxerutin effect on gastric ulcers induced by ketorolac in rats: Relation with oxidative stress

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ABSTRACT

Gastric ulcers are an essential side effect associated with the use of non-steroidal anti-inflammatory drugs (NSAIDs). Free radicals are one of the important mechanisms contributing to the development of gastric ulcers caused by NSAIDs. This prompted us to choose troxerutin, which has antioxidant and anti-inflammatory effects, especially with a lack of studies investigating the preventive effect of troxerutin on gastric ulcers. Twenty-nine rats were divided into five groups: A Vehicle group, a Keto group (30 mg/kg of ketorolac), and two troxerutin groups (150 mg/kg or 200 mg/kg of troxerutin, respectively). A Miso group was used as a reference with (100 μg/kg of misoprostol). Troxerutin and misoprostol were administered orally 1 h before ketorolac. The ulcer index was determined considering the numbers and severity of ulcerations. Gastric tissue inflammation was evaluation microscopically. Both thiobarbituric acid reactive substance levels and catalase activity were measured as markers of oxidative stress in gastric tissue. Our data showed an improvement in ulcer indices with troxerutin and misoprostol compared with ketorolac, with improvement in gastric inflammation observed with misoprostol but not with troxerutin. These results were accompanied by a reduction in gastric oxidative stress induced by ketorolac with both troxerutin and misoprostol. This study highlights, for the first time, the antioxidant effect of troxerutin on gastric ulcers. This effect may contribute to the good prevention of ketorolac-induced gastric ulcers.

1. Introduction

Peptic ulcer disease (PUD) is a common digestive disorder [1]. It is the damage to the inner lining of the digestive tract secondary to the secretion of gastric acid and pepsin [2]. In recent years, the leading cause of ulcers in many countries has shifted from Helicobacter pylori (H.pylori) infection to the use of non-steroidal anti-inflammatory drugs (NSAIDs) [3]. When causative agents such as gastric acid and NSAIDs overwhelm the mucosal defense, a gastric mucosal lesion such as gastric erosion and ulcer may develop [4].

Ketorolac is one of the NSAIDs which used for the short-term treatment of moderate to severe pain such as postoperative pain and musculoskeletal pain [5]. Its use has been associated with the potential for several serious side effects, such as gastrointestinal bleeding, perforated ulcers, and coagulation disorders [6]. NSAIDs are known to cause peptic ulcers through both local and systemic

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mechanisms. Local mechanisms involve the production of reactive oxygen species (ROS) such as superoxides, and free hydroxyl radicals. Systemic mechanisms involve the inhibition of prostaglandin production through inhibition of cyclooxygenases (COXs) [7], which inevitably impairs the mucosal defense mechanism [4,8]. NSAIDs cause increased adhesion of neutrophils to the lining of blood vessels, which is accompanied by the release of protease enzymes and free radicals derived from oxygen from these cells, and this leads to many endothelial and epithelial injuries [9].

There are many strategies to prevent or reduce gastrointestinal injuries induced by NSAIDs, such as proton pump inhibitors, H2-receptor antagonists, and Misoprostol (an analog of prostaglandin E1) [10]. However, the occurrence of severe adverse events limits the patient's quality of life [11]. Glutamine and Coenzyme Q10 are considered promising treatments for peptic ulcers due to their antioxidant properties [12,13]. In addition to phytotherapy, which contains different chemical groups such as alkaloids, terpenes, polysaccharides, and phenolic compounds, have proven their anti-inflammatory and antioxidant role in the management of peptic ulcer, as well as inhibiting acid secretion and enhancing defense factors [14].

As a result of the involvement of oxidative stress in causing peptic ulcers caused by NSAIDs, it was necessary to study new compounds that possess antioxidant properties to investigate their effectiveness in preventing gastric ulcers. Troxerutin is a semi-synthetic bioflavonoid derived from rutin, which has a preventive effect on gastric ulcers [15–17].

Studies have indicated that troxerutin has many pharmacological effects, such as an antioxidant effect. It has been proven to reduce levels of thiobarbituric acid reactive substances (TBARS) and improve the effectiveness of enzymatic antioxidants and levels of non-enzymatic antioxidants in many tissues [18–20]. It also has an anti-inflammatory effect by reducing inflammatory cytokine levels [21,22]. Other studies have indicated that it possesses anti-hyperlipidemic, anti-diabetic, and anti-tumor effect, and also has protective effects on the kidneys. It has an important role in the management of hemorrhoidal disease and chronic venous insufficiency. Troxerutin is highly absorbed from the gastrointestinal tract and exerts its protective effects without having a cytotoxic effect [23–25]. Despite numerous studies investigating the effects of troxerutin, its effects on the gastrointestinal system have not been thoroughly explored. While there are studies on the effect of troxerutin on ulcerative colitis [21], morphological intestinal mucosa changes induced by 5-flurouracil [26], and colon carcinogenesis [23], no study has investigated its role in the prevention of peptic ulcers, and this is what our study aims to achieve.

2. Results

2.1. Stomach macroscopic examination

Stomach macroscopic examination revealed the presence of white lesions with a diameter of 0.5–2 mm in both preventive groups and ketorolac-treatment group (keto), in addition to the presence of congestion, spot hemorrhages, and hemorrhagic streaks of 1–5 mm long.

2.1.1. Ulcer index according to method 1

(severity of gastric lesions was assessed depending on the length of lesions) was calculated as follows: Pretreatment with carboxymethyl cellulose (CMC) in the Vehicle group produced an ulcer index (UI) (0.45 ± 1), whereas ketorolac increased the ulcer index (2.35 ± 16) significantly ($p < 0.001$). Troxerutin (150 mg/kg) reduced the ulcer index (2.02 ± 10.6), with a preventive index (P.I) of (33.75 %), while the ulcer indices for the groups pretreated with troxerutin (200 mg/kg) and misoprostol were [(1.99 ± 12.17), ((2.12 ± 12.17, respectively, with a P.I = 23.93 % for each]. (Figs. 1 and 2).

2.1.2. Ulcer index according to method 2

(the severity of gastric lesions was assessed depending on the depth of lesions):

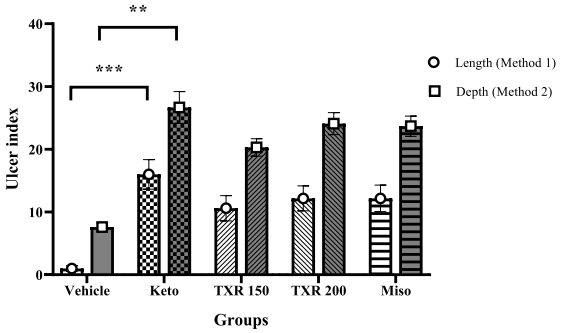

Fig. 1. The effect of troxerutin and misoprostol on ulcer index depending on lesion length [(UI = number of lesions × severity factor (from 0 to 3)] and depth [UI = number of ulcers per rat + severity score (from 1 to 4)+ (percentage of animals with ulcers/10). Troxerutin)150 mg/kg) reduced the ulcer index, but this decrease was slight when troxerutin (200 mg/kg) and misoprostol were given. Data were expressed as mean ± SEM, **$p < 0.01$, ***$p < 0.001$ (Keto vs Vehicle); (n = 5–6/group).

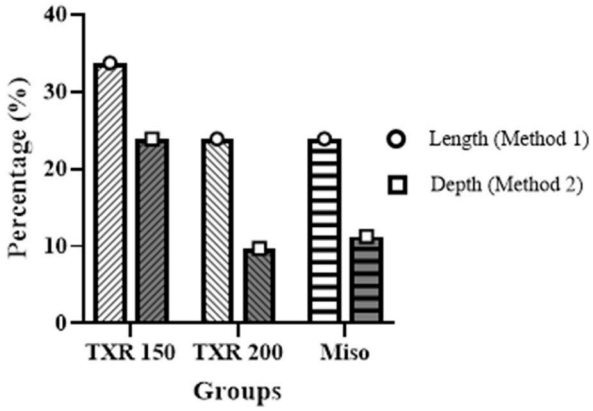

Fig. 2. The preventive index of troxerutin (150, 200) and the reference drug (misoprostol) depending on length and depth of lesions; (n = 5–6/group).

Ketorolac at a dose of 30 mg/kg caused an increase in the ulcer index (2.53 ± 26.67) significantly ($p < 0.01$) compared with the Vehicle group (0.68 ± 7.6). Troxerutin (150 mg/kg) reduced the ulcer index (1.39 ± 20.3) with a preventive index of (23.88 %), while the ulcer index with Troxerutin (200 mg/kg) was (1.75 ± 24.08), with P.I of (9.71 %). The ulcer index in the misoprostol group was (1.62 ± 23.67) with P. I of (11.24 %) (Figs. 1 and 2).

2.2. Stomach microscopic examination

2.2.1. Evaluation of inflammation in the stomach tissue

No inflammatory manifestation was observed in the stomach tissues of the Vehicle group, while focal inflammation signs were found in the lamina propria of the stomach tissues of rats treated with ketorolac, represented by neutrophils infiltration mainly. Troxerutin at a dose of 150 mg/kg slightly reduced neutrophils infiltration compared with the ketorolac group. While troxerutin (200 mg/kg) did not show any improvement. Misoprostol was able to completely prevent the neutrophils infiltration caused by ketorolac (Fig. 3). These effects are further illustrated in the detailed subfigures (Fig. 4(A–F)).

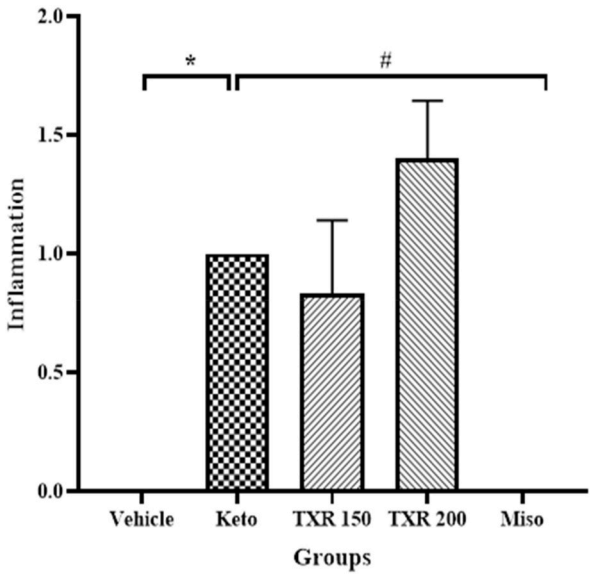

Fig. 3. The effect of troxerutin and misoprostol on inflammation in the stomach tissue. Both doses of troxerutin did not cause significant effect on ketorolac-induced inflammatory cell infiltration (neutrophils mainly), while misoprostol prevented it. Data were expressed as mean ± SEM, *p<0.05 (Keto vs Vehicle), #p<0.05 (Miso vs Keto); (n=5–6/group).

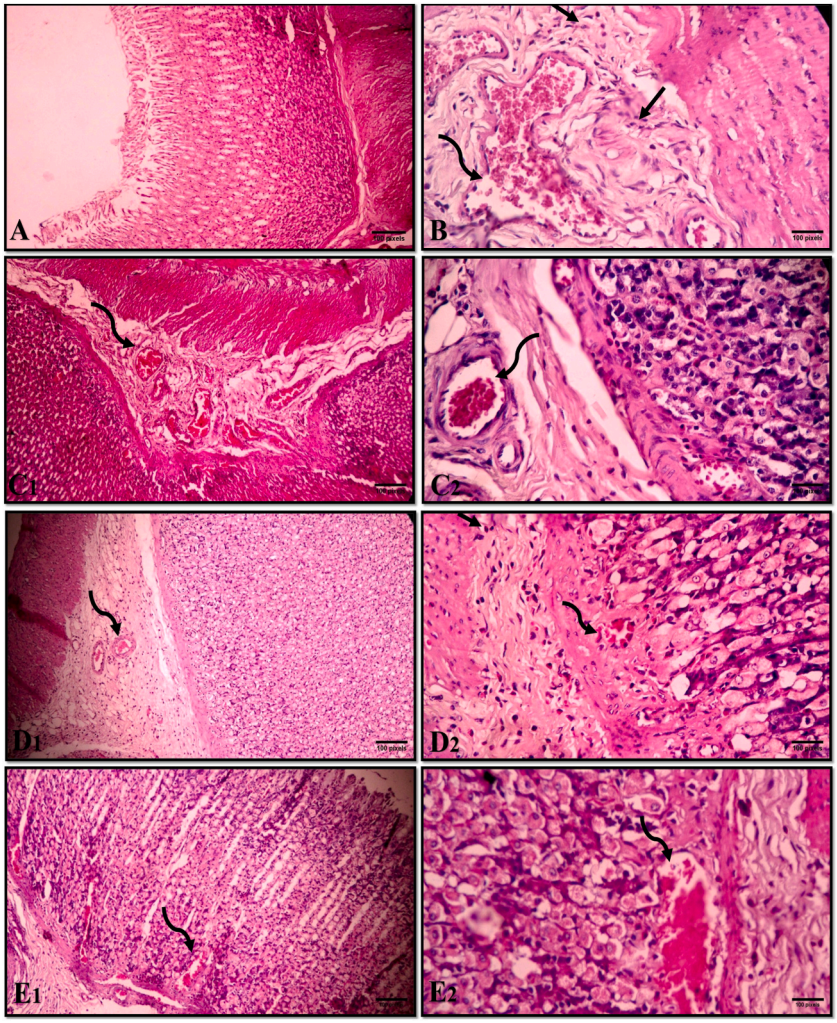

Fig. 4. Histological section of stomach tissue for different study groups. ↓: indicates the presence of inflammatory infiltrates, ↙: indicates the presence of congestion in the blood vessels. A: Vehicle (x100), B: Keto (x400), C1: TXR 150 (x100), C2: TXR 150 (x400), D1: TXR 200 (x100), D2: TXR 200 (x400), E1: Miso (x100), E2: Miso (x400). The inflammation was evaluated according to the following scale: 0: No inflammation, 1: Focal inflammation of the lamina propria, 2: Diffused inflammation of the lamina propria, 3: Focal inflammation of the muscle layer, 4: Diffused inflammation in the muscle layer.

2.3. Measurement of oxidative stress biomarkers in the stomach tissues

2.3.1. Measurement of lipid peroxidation products levels

The ketorolac treated group didn't show any significant difference (62.71 ± 5.77) compared with the Vehicle group (71.92 ± 6.812) regarding TBARS. Troxerutin pretreatment at both doses (150, 200 mg/kg) significantly reduced TBARS levels in the stomach tissue [(32.05 ± 3.85), ($p < 0.001$); (38.78 ± 4.58), ($p < 0.01$), respectively] compared with the ketorolac group (62.71 ± 5.77). pretreatment with misoprostol was also effective in reducing TBARS levels [(43.91 ± 3.748), $p < 0.05$ compared with ketorolac group] with no difference noticed when comparing with troxerutin groups (Fig. 5).

2.3.2. Measurement of catalases activity

Ketorolac significantly decreased the catalase activity [5.428 ± 42.25; $p < 0.001$] compared with the Vehicle group (12.33 ± 84). Pretreatment with troxerutin (150 mg/kg) significantfly reversed the decrease of catalase activity in the stomach tissue caused by ketorolac (4.924 ± 68.5); ($p < 0.05$). Both troxerutin (200 mg/kg) and misoprostol didn't show any improvement in the stomach catalase activity (Fig. 6).

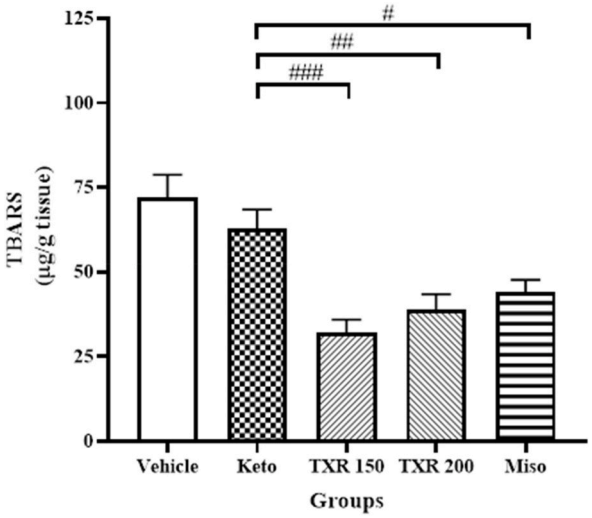

Fig. 5. The effect of troxerutin and misoprostol on TBARS levels in stomach tissue. Preventive treatments reduced significantly TBARS levels compared with the ketorolac group. Data were expressed as mean ± SEM, $^{\#}P < 0.05$ (Miso vs Keto), $^{\#\#}P < 0.01$ (TXR 200 vs Keto), $^{\#\#\#}P < 0.001$ (TXR 150 vs Keto); (n = 5–6/group).

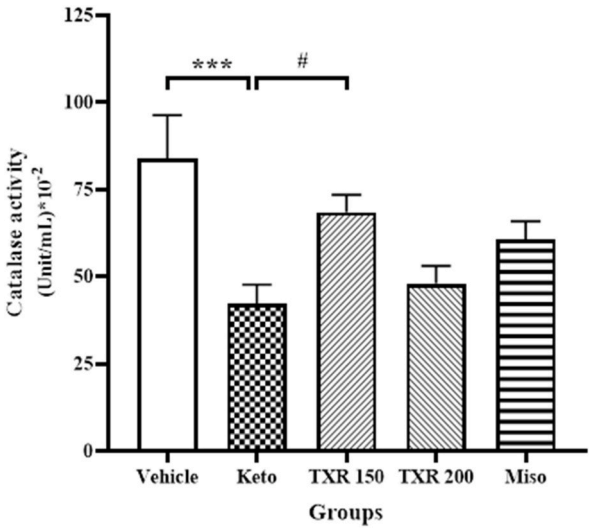

Fig. 6. The effect of troxerutin and misoprostol on catalase activity in stomach tissue. Troxerutin)150 mg/kg) increased tissue catalase activity, which decreased as a result of ketorolac administration.Data were expressed as mean ± SEM, ***P < 0.001 (Keto vs Vehicle), $^{\#}P < 0.05$ (TXR 150 vs Keto); (n = 5–6/group).

3. Discussion

Ketorolac possesses analgesic, anti-inflammatory, and antipyretic effects as one of the NSAIDs [27]. However, it has been associated with cardiovascular, renal, and gastrointestinal risks [5]. The major mechanism of action of ketorolac in causing damage to the gastric mucosa depends on the inhibition of the synthesis of prostaglandins by inhibiting both COX enzymes and thus causing impairment of mucosal defense. NSAIDs also have a role in increasing the adhesion of neutrophils to the vascular endothelium through their role by inhibiting the enzyme COX 2 mainly and thus the release of proteases and free radicals which mediate many of the injuries associated with NSAIDs [9].

Therefore, the modulation of oxidative stress represents an evidence-based choice for the management of gastrointestinal disorders [28]. Several studies have proven the role of troxerutin as an antioxidant in the treatment and prevention of several diseases such as Ulcerative colitis [21], cisplatin-induced kidney injury [19], and it has an anti-cancer effect [29]. In this study, we aimed to investigate the preventive effect of troxerutin on gastric ulcers induced by ketorolac, compared with misoprostol whose protective effect of gastric ulcers had already been proven. It is important to note that this study is the first to address the effects of troxerutin on gastric ulcers.

First of all, our study showed a negative effect of ketorolac on gastric tissue in experimental rats. As it led to ulcers in the stomach tissue observed macroscopically, with an increase of the ulcer index (which was calculated by two methods depending on the length and depth of lesions), these macroscopic observations accompanied by the microscopic study showed focal inflammation in the lamina propria represented by neutrophil infiltration to the stomach tissue. This ulcer-inducing effect of ketorolac and other NSAIDs has been previously demonstrated by the microscope study in several animal studies [15,30] and humans [31].

Neutrophils infiltration following their adhesion to the lining of blood vessels is an early event in gastric damage induced by NSAIDs, as studies have proven their involvement in gastric damage induced by NSAIDs, as they cause the production of many factors such as proteolytic enzymes and reactive oxygen species. Therefore, studying the parameters of oxidative stress is a reasonable way to understand mechanisms causing gastric damage induced by NSAIDs [15,32,33].

In our study, the gastric damage caused by ketorolac administration was associated with a decrease in the activity of catalase enzymes in the gastric tissue, indicating a role of oxidative stress in causing gastric ulcers induced by ketorolac. This result was consistent with the study of Shaik and colleagues in 2022, which showed a decrease in the activity of tissue catalase enzymes and the role of oxidative stress in gastric ulcers induced by NSAIDs in experimental rats [34]. While our study differed with it in terms of the fact that tissue TBARS levels were not affected by the administration of ketorolac, while its tissue levels increased in the study of Shaik et al. This difference in the results of TBARS levels may be due to a difference in the compound used to induce gastric ulcers, as Shaik's study used indomethacin compound.

The unchanged levels of TBARS, although the activity of catalase enzymes decreased in the stomach tissue as a result of ketorolac administration, may be due to the fact that catalases hydrolyze H_2O_2 in the tissue into O_2 and H_2O, and the decrease in its activity may lead to the production of large amounts of Hydroxyl radicals OH. According to the Fenton reaction [35], these large amounts of free hydroxyl radicals cause direct damage to the gastric cell membranes through the process of lipid peroxidation, which leads to high levels of TBARS, the by-product of this process. In our study, ketorolac may have decreased the activity of catalase enzymes to a lesser extent than that which cause an increase in TBARS levels in the stomach tissue. Therefore, the damage was not at the level of damage to membrane lipids.

In the current study, troxerutin played a good role in the prevention of gastric ulcers induced by ketorolac, at both doses (150 and 200 mg/kg) leading to a clear decrease in the ulcer index (length and depth of lesions) and also had an obvious effect on the preventive index, in particular, at a dose of (150 mg/kg), while it had a slight effect at the higher dose (200 mg/kg). The effect of troxerutin was not fully evident on the gastric tissue inflammation, and there was no clear improvement in the infiltration of neutrophils observed when ketorolac was given alone. Our results were consistent with the study of Abdel-Raheem where rutin (Troxerutin-like compound) led to a significant decrease in ulcer index induced by indomethacin, while it did not match with this study in terms of histological study, which proved an important decrease in the inflammatory manifestations caused by indomethacin in gastric tissue when treated with rutin. Similarly to the precedent researches on the gastric ulcers induced by NSAIDs [36–38], misoprostol -our reference medicament-proved its ability to prevent the gastric ulcers induced by ketorolac and reduced the ulcer index, neutrophil infiltration, and histological damage of the stomach.

The antioxidant effect of troxerutin was similar to that observed by misoprostol, which the preventive effects of misoprostol on gastrointestinal tract damages partially attributed to its antioxidant effects [37]. which was confirmed by our study.

In our study, troxerutin in both doses had a good antioxidant effect caused by ketorolac in the gastric tissue shown by reducing TBARS levels and increasing catalase activity (at a dose 150 mg/kg). This confirms its effectiveness as an antioxidant indicated in several previous studies in renal and colon tissues [19,21], which may contribute to the troxerutin protective effect on the gastric ulcers induced by ketorolac.

In addition to the anti–oxidant effect, troxerutin has anti-inflammatory and anti-apoptotic effects [21,39] that may also contribute to the prevention of NSAIDs-induced peptic ulcers. Neutrophil infiltration leads to the production of inflammatory cytokines such as tumor necrosis factor-alpha [40]. Apoptosis may also contribute to the development of stomach ulcers and is related to the inflammatory response and oxidative stress [41]. It may also play a role in regulating acid secretion pathways, like other flavonoids [40]. In general, we need to conduct further studies to determine the other mechanisms of action which may contribute to the preventing effect of troxerutin on peptic ulcers induced by NSAIDs.

4. Materials and methods

4.1. Aim of the study

The importance of our study comes from the absence of previous studies that revealed the role of troxerutin in the prevention of gastric ulcers induced by NSAIDs.

4.2. Drugs

Troxerutin, as a yellow powder, was obtained from Mediotec for Pharmaceutical Industries (Homs, Syria). Ketorolac, as a product, was purchased from Avenzor for Pharmaceutical Industries (Rural Damascus, Syria). Misoprostol, as a product, was purchased from Hama Pharma for Pharmaceutical Industries (Hama, Syria).

4.3. Experimental design

4.3.1. Animals

Twenty-nine wistar albino rats of (10–12) weeks of age and (100–245 g) weight were provided by the Atomic Energy Authority-Damascus-Syria. Animals were housed in groups of three rats per cage at the experimental animal incubators, Faculty of Pharmacy-Damascus university. All animals were acclimatized for 7 days before starting the experiments to prevent or minimize stress in a new environment. The animals were kept under controlled conditions of temperature (25 ± 2 °C) and humidity, with a 12 h light/dark cycle and access to water and food granules. The guidelines of ethics were followed in dealing with animals, in terms of preserving the dignity of animals, alleviating their suffering, and reducing the number to a minimum. The study was conducted in accordance with the guidelines for Good Laboratory Practices in Non-Clinical Studies, and the experimental procedure was approved by the Biomedical Research Ethics Committee at Damascus University (ID: PH-210224-207/Feb 21, 2024).

4.3.2. Animals grouping

Rats were randomly divided into five groups, each consisting of (5–6) rats. Animals were fasted for 24 h before the study but had free access to water.

One group represented the Vehicle control group (Vehicle), which received only CMC solution (1 %, orally). The second group represented the positive control group (Keto) received a single dose of ketorolac (30 mg/kg, orally) (produced significant gastric damage [30]). The third and fourth groups represented the preventive groups (TXR 150, 200) that received a single dose of troxerutin (150, 200 mg/kg respectively, orally) (these doses have been shown to prevent and treat many diseases in which oxidative stress plays a role [22,42–45]), 1 h before a ketorolac administration (30 mg/kg, orally). The reference group (Miso) received a single dose of misoprostol (100 μg/kg, orally), 1 h before a ketorolac administration (an effective dose produced protection against diclofenac-induced damage to gastric mucosa [36]). (Fig. 7).

4.4. Samples collection and processing

At the end of the experiment, 18 h after drugs administration, rats were sacrificed, and each stomach was isolated and opened along the greater curvature, rinsed with saline to remove gastric contents and blood clots and examined by a 10x magnifier lens to assess the formation of ulcers. Then one portion of each stomach was taken and preserved at −80 °C for later titrations, while the rest was kept in 10 % formalin for histological study.

4.5. Measured parameters

4.5.1. Ulcer index

The stomach was examined, and the gastric ulcers were assessed via two macroscopic methods, depending on either the length of the lesions or the depth and severity of the lesions.

Method 1:

The severity of gastric lesions was assessed depending on the length of lesions according to the following scale [46]:

Severity factor (0): no lesion,

Severity factor (1): lesions of <2 mm in length,

Severity factor (2): lesions of 2–4 mm length,

Severity factor (3): lesions of >4 mm length.

The ulcer index was calculated for each rat by multiplying the number of lesions by the severity factor and then calculating the mean of each group.

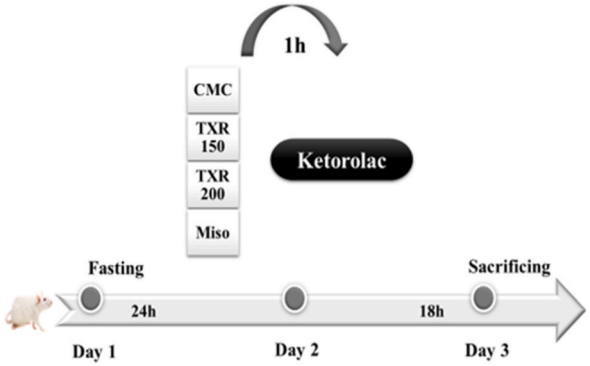

Fig. 7. Experiment design.
Excipient (CMC) or prophylactic drugs were given 1 h before ulcer induction by ketorolac (administered 24 h after fasting). Rats were sacrificed 18 h after ulcer induction.

Method 2:

The severity of gastric lesions was assessed depending on the depth of lesions, and the ulcer index was calculated for each rat according to the following scale [47]:

0: Normal colored stomach,
0.5: Red coloration,
1: Spot ulcer,
1.5: hemorrhagic streak,
2: deep ulcers,
3: Perforation.

Ulcer index (UI) = UN + US + (UP/10)

UN: number of ulcers per rat.
US: severity score.
UP: percentage of animals with ulcers.

4.5.2. Preventive index: [47,48]

P.I = (UI of ulcerated group - UI of pretreated group) x 100 / UI of ulcerated group.

4.5.3. Histopathological examination

The histological study was carried out in the histopathology laboratory at the Faculty of Dentistry - University of Damascus. Briefly, gastric tissue samples were fixed in 10 % formaldehyde, followed by dehydration using ascending alcohol concentrations (70 %, 95 % and 100 %). After xylene clearance, the processed tissues were infiltrated and embedded in paraffin blocks. Subsequently, 4 μm thick sections were obtained using a microtome. Finally, the sections were stained with hematoxylin and eosin and examined under an Olympus light microscope. The inflammation was evaluated according to the following scale [49].

0: No inflammation,
1: Focal inflammation of the lamina propria,
2: Diffused inflammation of the lamina propria,
3: Focal inflammation of the muscle layer,
4: Diffused inflammation in the muscle layer.

The inflammation severity score was calculated for each rat, and the mean was taken for each group. The histological images were captured at 100x and 400× magnifications.

4.5.4. Biochemical assays

A homogenate was prepared from gastric tissue by adding phosphate-buffered saline (PBS; pH = 7.4), and homogenization was performed with an Ultrasonic Processor-Cole-parmer.

4.5.4.1. Lipid peroxidation product levels. The levels of TBARS were estimated in gastric tissue according to Patil et al. [50]. the working solution was prepared as follows: 0.375 % thiobarbituric acid (TBA), 15 % trichloroacetic acid (TCA), and 0.25N hydrochloric acid. The volume was completed to 100 ml with distilled water. 500 μL of homogenate and 2000 μL of reagent were added, and the mixture was placed in boiling water for 15 min. After cooling, the samples were centrifuged (3000 rpm for 10 min) at room temperature. Finally, the absorbance of the supernatant was measured by a spectrophotometer at a λ = 532 nm and it was expressed as (μg/g tissue).

4.5.4.2. Catalase enzymes activity. Catalase activity was manually measured in gastric tissue according to Cohen et al. [51]. Gastric tissue homogenate was centrifuged at (3600 rpm for 10 min). 0.5 ml of the supernatant was added to the test tubes. The reaction was started by adding 5 ml of hydrogen peroxide H_2O_2 (30 mM). Stop the reaction after 3 min by adding 1 ml of H_2SO_4 (3M). Finally, 7 ml of potassium permanganate ($Kmno_4$; 0.01M) was added. The absorbance was measured by a spectrophotometer at λ = 480 nm over 30–60 s.

Catalase Enzyme activity = Absorbance*V*1000/min*M*v*Y

V: total volume of the reaction mixture,
M: molar extinction coefficient (M = 40),
V: volume of sample used,
Y: tissue weight.

4.6. Statistical analysis

Data were expressed as mean ± SEM of n observations, where n represents the number of animals or samples. For oxidative stress

biomarkers, one-way analysis of variance (ANOVA) and Dunnett's multiple comparisons as a post hoc test were used. For the study of ulcer index and histological studies, Kruskal–Wallis test followed by Dunn's multiple comparisons test was used. Statistical significance was set at $p < 0.05$ (by GraphPad Prism software version 8).

5. Conclusion

Our study is pioneering in proving the capacity of troxerutin to reduce the gastric oxidative stress induced by ketorolac, which gives new hope for adding new effective compounds useful in the prevention of ketorolac-induced gastric ulcers, with a relatively high margin of safety. Additional studies with different doses of troxerutin (<150 mg/kg) will be useful in the future.

Additional information

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Data availability statement

Data included in the article.

Ethics statement

Ethical approval for this study was obtained from the Biomedical Research Ethics Committee at Damascus University (ID: PH-210224-207/Feb 21, 2024). We followed all guidelines and regulations during this study.

CRediT authorship contribution statement

Alaa Zouher Darkazally: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Amirah Alnour:** Writing – review & editing, Visualization, Investigation. **Shadi Homsi:** Writing – review & editing, Supervision, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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List of abbreviations:

- CMC Carboxymethyl cellulose
- COXs Cyclooxygenases
- *H. pylori* Helicobacter pylori
- Keto Ketorolac-treatment group
- NSAIDS Non-steroidal anti-inflammatory drugs
- P.I: Preventive index
- PBS Phosphate buffer saline
- PUD Peptic ulcer disease
- ROS Reactive oxygen species
- TBA Thiobarbituric acid
- TBARS Thiobarbituric acid reactive substance
- TCA Trichloroacetic acid
- TXR Troxerutin-treatment group
- UI Ulcer index

References

[1] J. Ren, X. Jin, J. Li, R. Li, Y. Gao, J. Zhang, X. Wang, G. Wang, The global burden of peptic ulcer disease in 204 countries and territories from 1990 to 2019: a systematic analysis for the Global Burden of Disease Study 2019, Int. J. Epidemiol. (2022), https://doi.org/10.1093/ije/dyac033.

[2] M. Narayanan, K.M. Reddy, E. Marsicano, Peptic ulcer disease and Helicobacter pylori infection, Mo, Méd. 115 (2018) 219–224, https://doi.org/10.1097/00001574-199208010-00019.

[3] X. Xie, K. Ren, Z. Zhou, C. Dang, H. Zhang, The global, regional and national burden of peptic ulcer disease from 1990 to 2019: a population-based study, BMC Gastroenterol. 22 (2022) 1–13, https://doi.org/10.1186/s12876-022-02130-2.

[4] H. Zatorski, Pathophysiology and risk factors in peptic ulcer disease, Introd. to Gastrointest, Dis. 2 (2017) 7–20, https://doi.org/10.1007/978-3-319-59885-7_2.

[5] N. Vadivelu, A.M. Gowda, R.D. Urman, S. Jolly, V. Kodumudi, M. Maria, R. Taylor, J.V. Pergolizzi, Ketorolac tromethamine - routes and clinical implications, Pain Pract. 15 (2015) 175–193, https://doi.org/10.1111/papr.12198.

[6] J.S. Gaynor, Other drugs used to treat pain, Handb. Vet. Pain Manag (2009) 260–276, https://doi.org/10.1016/B978-032304679-4.10014-0.

[7] C. Musumba, D.M. Pritchard, M. Pirmohamed, Review article: cellular and molecular mechanisms of NSAID-induced peptic ulcers, Aliment. Pharmacol. Ther. 30 (2009) 517–531, https://doi.org/10.1111/j.1365-2036.2009.04086.x.

[8] S.C. Park, H.J. Chun, C.D. Kang, D. Sul, Prevention and management of non-steroidal anti-inflammatory drugs-induced small intestinal injury, World J. Gastroenterol. 17 (2011) 4647–4653, https://doi.org/10.3748/wjg.v17.i42.4647.

[9] J.L. Wallace, Pathogenesis of NSAID-induced gastroduodenal mucosal injury, Best Pract. Res. Clin. Gastroenterol. 15 (2001) 691–703, https://doi.org/10.1053/bega.2001.0229.

[10] M. Drini, Peptic ulcer disease and non-steroidal anti-inflammatory drugs, Aust. Prescr. 40 (2017) 91–93, https://doi.org/10.18773/austprescr.2017.037.

[11] F.L. Fagundes, Q.C. Pereira, M.L. Zarricueta, R. de C. Dos Santos, Malvidin protects against and repairs peptic ulcers in mice by alleviating oxidative stress and inflammation, Nutrients 13 (2021), https://doi.org/10.3390/nu13103312.

[12] A.M. Malash, D.M. Abdallah, A.M. Agha, S.A. Kenawy, Gastroprotective efficacy of Coenzyme Q10 in indomethacin-induced gastropathy : other potential mechanisms, 2012. https://doi.org/10.1155/2012/957898, 2012.

[13] S. Okabe, K. Takeuchi, K. Nakamura, K. Takagi, Inhibitory Effects of L-Glutamine on the Aspirin-Induced Gastric Lesions in the Rat, 1974, pp. 605–611.

[14] A.S. Awaad, R.M. El-Meligy, G.A. Soliman, Natural products in treatment of ulcerative colitis and peptic ulcer, J. Saudi Chem. Soc. 17 (2013) 101–124, https://doi.org/10.1016/J.JSCS.2012.03.002.

[15] I.T. Abdel-Raheem, Gastroprotective effect of rutin against indomethacin-induced ulcers in rats, Basic Clin. Pharmacol. Toxicol. 107 (2010) 742–750, https://doi.org/10.1111/j.1742-7843.2010.00568.x.

[16] Y. Liu, L. Gou, X. Fu, S. Li, N. Lan, X. Yin, Protective effect of rutin against acute gastric mucosal lesions induced by ischemia-reperfusion, Pharm. Biol. 51 (2013) 914–919, https://doi.org/10.3109/13880209.2013.771375.

[17] C.S. Jeong, Evaluation for protective effect of rutin, a natural flavonoid, against Hcl/ethanol-induced gastric lesions, Biomol. Ther. 17 (2009) 199–204, https://doi.org/10.4062/biomolther.2009.17.2.199.

[18] B. Raja, D. Saranya, R. Prabhu, Role of flavonoid troxerutin on blood pressure, oxidative stress and regulation of lipid metabolism, Front. Biosci. - Elit. 11 (2019) 121–129, https://doi.org/10.2741/E851.

[19] F. Dehnamaki, A. Karimi, A.A. Pilevarian, I. Fatemi, E. Hakimizadeh, A. Kaeidi, M. Allahtavakoli, Treatment with troxerutin protects against cisplatin-induced kidney injury in mice, Acta Chir. (2018) 1–7, https://doi.org/10.1080/00015458.2018.1455418. Belg. 0.

[20] R. Badalzadeh, L. Chodari, V. Ghorbanzadeh, Troxerutin, a bioflavonoid, improves oxidative stress in blood of streptozotocin-induced Type-1 diabetic rats, Iran, J. Pharmaceut. Sci. 13 (2017) 75–86, https://doi.org/10.22034/ijps.2017.31148.

[21] X. Wang, Y. Gao, L. Wang, D. Yang, W. Bu, L. Gou, J. Huang, X. Duan, Y. Pan, S. Cao, Z. Gao, C. Cheng, Z. Feng, J. Xie, R. Yao, Troxerutin improves dextran sulfate sodium-induced ulcerative colitis in mice, J. Agric. Food Chem. 69 (2021) 2729–2744, https://doi.org/10.1021/acs.jafc.0c06755.

[22] S.A. Salama, H.H. Arab, I.A. Maghrabi, Troxerutin down-regulates KIM-1, modulates p38 MAPK signaling, and enhances renal regenerative capacity in a rat model of gentamycin-induced acute kidney injury, Food Funct. 9 (2018) 6632–6642, https://doi.org/10.1039/c8fo01086b.

[23] R. Vinothkumar, R. Vinoth Kumar, V. Karthikkumar, P. Viswanathan, J. Kabalimoorthy, N. Nalini, Oral supplementation with troxerutin (trihydroxyethylrutin), modulates lipid peroxidation and antioxidant status in 1,2-dimethylhydrazine-induced rat colon carcinogenesis, Environ. Toxicol. Pharmacol. 37 (2014) 174–184, https://doi.org/10.1016/j.etap.2013.11.022.

[24] M. Zamanian, G. Bazmandegan, A. Sureda, E. Sobarzo-Sanchez, H. Yousefi-Manesh, S. Shirooie, The protective roles and molecular mechanisms of troxerutin (vitamin P4) for the treatment of chronic diseases: a mechanistic review, Curr. Neuropharmacol. 19 (2020) 97–110, https://doi.org/10.2174/1570159x18666200510020744.

[25] Z. Ahmadi, R. Mohammadinejad, S. Roomiani, E.G. Afshar, M. Ashrafizadeh, Biological and therapeutic effects of troxerutin: molecular signaling pathways come into view, J. Pharmacopuncture 24 (2021) 1–13, https://doi.org/10.3831/KPI.2021.24.1.1.

[26] J.A.L. de Miranda, C. da S. Martins, L. de S. Fideles, M.L.L. Barbosa, J.E.F. Barreto, H.B. Pimenta, F.O.R. Freitas, P.V. de S. Pimentel, C.S. Teixeira, A.G. Scafuri, M.C.D.S. Luciano, J.L. Araújo, J.A. Rocha, I.G.P. Vieira, N.M.P.S. Ricardo, M. da S. Campelo, M.E.N.P. Ribeiro, G.A. de C. Brito, G.S. Cerqueira, Troxerutin prevents 5-fluorouracil induced morphological changes in the intestinal mucosa: role of cyclooxygenase-2 pathway, Pharmaceuticals 13 (2020), https://doi.org/10.3390/ph13010010.

[27] J. Gillis, R. Brogden, Ketorolac | SpringerLink, (n.d.). https://link.springer.com/article/10.2165/00003495-199753010-00012 (accessed March 12, 2023).

[28] R. Vona, L. Pallotta, M. Cappelletti, C. Severi, P. Matarrese, The impact of oxidative stress in human pathology: focus on gastrointestinal disorders, Antioxidants 10 (2021) 1–26, https://doi.org/10.3390/antiox10020201.

[29] N. Susan, K. George, A. Alias, A. Selvam, Toxicology in Vitro Anticancer mechanism of troxerutin via targeting Nrf2 and NF- κ B signalling pathways in hepatocarcinoma cell line, Toxicol. Vitro 54 (2019) 317–329, https://doi.org/10.1016/j.tiv.2018.10.018.

[30] J.L. Wallace, W. McKnight, B.K. Reuter, N. Vergnolle, NSAID-Induced gastric damage in rats: requirement for inhibition of both cyclooxygenase 1 and 2, Gastroenterology 119 (2000) 706–714, https://doi.org/10.1053/gast.2000.16510.

[31] L.L. Estes, D.W. Fuhs, A.H. Heaton, C.S. Butwinick, Gastric ulcer perforation associated with the use of injectable ketorolac, Ann. Pharmacother. 27 (1993) 42–43, https://doi.org/10.1177/106002809302700111.

[32] H. Asako, P. Kubes, J. Wallace, R.E. Wolf, D.N. Granger, Modulation of leukocyte adhesion in rat mesenteric venules by aspirin and salicylate, Gastroenterology 103 (1992) 146–152, https://doi.org/10.1016/0016-5085(92)91107-F.

[33] M.D. Jiménez, M.J. Martín, C.A. De La Lastra, L. Bruseghini, J.M. Herrerías, V. Motilva, Role of L-arginine in ibuprofen-induced oxidative stress and neutrophil infiltration in gastric mucosa, Free Radic. Res. 38 (2004) 903–911, https://doi.org/10.1080/10715760410001705168.

[34] R.A. Shaik, B.G. Eid, Piceatannol affects gastric ulcers induced by indomethacin: association of antioxidant, anti-inflammatory, and angiogenesis mechanisms in rats, Life 12 (2022) 356, https://doi.org/10.3390/LIFE12030356, 12 (2022) 356.

[35] A. Abdeen, M. Aboubakr, D. Elgazzar, M. Abdo, A. Abdelkader, S. Ibrahim, A. Elkomy, Rosuvastatin attenuates piroxicam-mediated gastric ulceration and hepato-renal toxicity in rats, Biomed, Pharma 110 (2019) 895–905, https://doi.org/10.1016/j.biopha.2018.11.004.

[36] E. El-Deen, N. El-Mahdy, M. Rashidy, M. Ghorab, S. Gad, H. Yassin, Diclofenac-induced gastric ulceration in rats: protective roles of pantoprazole and misoprostol, Br. J. Pharmaceut. Res. 11 (2016) 1–12, https://doi.org/10.9734/bjpr/2016/24636.

[37] J.H. Ahmed, A. Jala, a A. Salman Al-Ahmed, E.A. Al-Masoodi, Evaluation of the gastroprotective effect of misoprostol, chitosan and their combination on indomethacin induced gastric ulcer in rats, Med. J. Basrah Univ. 29 (2011) 1–8, https://doi.org/10.33762/mjbu.2011.49471.

[38] G. Ateufack, E.C. Domgnim Mokam, M. Mbiantcha, R.B. Dongmo Feudjio, N. David, A. Kamanyi, Gastroprotective and ulcer healing effects of piptadeniastrum Africanum on experimentally induced gastric ulcers in rats, BMC Compl. Alternative Med. 15 (2015) 1–10, https://doi.org/10.1186/S12906-015-0713-5/TABLES/4.

[39] N.A. Panat, D.K. Maurya, S.S. Ghaskadbi, S.K. Sandur, Troxerutin, a plant flavonoid, protects cells against oxidative stress-induced cell death through radical scavenging mechanism, Food Chem. 194 (2016) 32–45, https://doi.org/10.1016/j.foodchem.2015.07.078.
[40] W. Zhang, Y. Lian, Q. Li, L. Sun, R. Chen, X. Lai, Z. Lai, E. Yuan, S. Sun, Preventative and therapeutic potential of flavonoids in peptic ulcers, Molecules 25 (2020), https://doi.org/10.3390/MOLECULES25204626.
[41] C. Serafim, M.E. Araruna, E. Alves Júnior, M. Diniz, C. Hiruma-Lima, L. Batista, A review of the role of flavonoids in peptic ulcer (2010–2020), Molecules 25 (2020) 5431, https://doi.org/10.3390/MOLECULES25225431.
[42] T. Baluchnejadmojarad, N. Jamali-Raeufy, S. Zabihnejad, N. Rabiee, M. Roghani, Troxerutin exerts neuroprotection in 6-hydroxydopamine lesion rat model of Parkinson's disease: possible involvement of PI3K/ERβ signaling, Eur. J. Pharmacol. 801 (2017) 72–78, https://doi.org/10.1016/j.ejphar.2017.03.002.
[43] Y. Yu, G. Zheng, Troxerutin protects against diabetic cardiomyopathy through NF-κB/AKT/IRS1 in a rat model of type 2 diabetes, Mol. Med. Rep. 15 (2017) 3473–3478, https://doi.org/10.3892/mmr.2017.6456.
[44] T. Guan, Y. Zheng, S. Jin, S. Wang, M. Hu, X. Liu, S. Huang, Y. Liu, Troxerutin alleviates kidney injury in rats via PI3K/AKT pathway by enhancing MAP4 expression, Food Nutr. Res. 66 (2022) 1–14, https://doi.org/10.29219/fnr.v66.8469.
[45] Z.Z. Oskuye, F.M. Bavil, G.R. Hamidian, K. Mehri, M. Ahmadi, H. Oghbaei, A.M. Vatankhah, R. Keyhanmanesh, Troxerutin affects the male fertility in prepubertal type 1 diabetic male rats. https://doi.org/10.22038/ijbms.2018.32678.7814, 2018.
[46] B.M. Peskar, K. Ehrlich, B.A. Peskar, Role of ATP-sensitive potassium channels in prostaglandin-mediated gastroprotection in the rat, J. Pharmacol. Exp. Therapeut. 301 (2002) 969–974, https://doi.org/10.1124/jpet.301.3.969.
[47] V. Prasanth Reddy, G. Sudheshna, S.K. Afsar, S. Sai Saran, S. Nelson Kumar, C. Raja Ram, K. Ravindra Reddy, Evaluation of anti-ulcer activity of Citrullus colocynthis fruit against pylorus ligation induced ulcers in male wistar rats, Int. J. Pharm. Pharmaceut. Sci. 4 (2012) 446–451.
[48] I.Z.A. Abdallah, H.A.H. Khattaba, G.H. Heebab, Gastroprotective effect of Cordia Myxa L. fruit extract against indomethacin-induced gastric ulceration in rats, Life Sci. J. 8 (2011) 433–445.
[49] S. Simões, R. Lopes, M.C.D. Campos, M.J. Marruz, M.E.M. da Cruz, L. Corvo, Animal models of acute gastric mucosal injury: macroscopic and microscopic evaluation, Anim. Model. Exp. Med. 2 (2019) 121–126, https://doi.org/10.1002/ame2.12060.
[50] N.R. Patil, V.P. Rasal, R.H. Malabade, Screening of Mandarin oil on indomethcin induced inflammatory bowel disease in wistar rats, Indian J. Pharm. Educ. Res. 48 (2014) 1–6, https://doi.org/10.5530/ijper.48.4s.1.
[51] G. Cohen, D. Dembiec, J. Marcus, Measurement of catalase activity in tissue extracts, Anal. Biochem. 34 (1970) 30–38, https://doi.org/10.1016/0003-2697(70)90083-7.