Journal of Chemistry and Nutritional Biochemistry
https://jcnb.sabapub.com
E ISSN: 2709-5932

2023 Volume 4, Issue 2: 51–72
DOI: https://doi.org/10.48185/jcnb.v4i2.951

The protective effects of betanin against experimental gastric ulcer by reduction of ROS and suppression of inflammatory genes via NF-κB, iNOS, COX-2 and TNF-α pathways

Othman A. Alghamdi*

Department of Biological Sciences, College of Science, University of Jeddah, Jeddah 22233, Kingdom of Saudi Arabia.

Received: 20.10.2023 • Accepted: 29.11.2023 • Published: 01.12.2023 • Final Version: 01.12.2023

Abstract: Betanin, a prominent compound found in beetroot, however, the potential protective effects of this compound against gastric damage and ulceration have yet to be explored. This study aims to investigate the impact of purified beetroot betanin (Bet) on the activities of inflammatory enzymes and gene expressions associated with inflammation in gastric tissues. Gastric ulcer rats were induced by ethanol at dose 5ml/kg body weight. Ulcerated rats were treated by purified betanin or omeprazole; from which some inflammation-related genes and gastric-inflammation enzymes were investigated using RT-PCR and biochemical analysis, respectively. Bet effectively suppressed the activities of key enzymes involved in gastric mucosal inflammation, including 5-Lipoxygenase (5-LO), Hyaluronidase (HAase), and myeloperoxidase (MPO), by 58%, 50%, and 45%, respectively. Docking studies showed Bet binding to catalytic sites or key regions for enzymes' activities, thus explaining the inhibitory capacity. Bet ingestion decreased the expression levels of inflammation-related genes, factor-kappa B (NF-ϰB) by 57%. This down-regulation subsequently led to a reduction in the expression of inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), and tumour necrosis factor-α (TNF-α). Bet ingestion supressed thiobarbituric acid reactive substance (TBARS) rates by 59%, augmented nitric oxide (NO) level by 107% and protected gastric tissues from damage and ulceration. Bet revealed gastro-protective effects as confirmed by a reduction in the ulceration surface area and the acidity by 75.9% and 40%, respectively, and a significant increase in the weight of mucus and the curative index by 117% and 74.5%, respectively. In conclusion, these outcomes of physiological and molecular-based investigations are indicative of potential use of Bet as a functional food additive for the prevention of gastric ulcer.

Keywords: ulcer, betanin, pigment, inflammation, gene expression

*Corresponding author: oalghamdi@uj.edu.sa

1. Introduction

One of the most prevalent illnesses of the digestive tract in humans is gastric ulcer (GU), which has an incidence rate of 10% of the global population [1]. One of the most frequent factors leading to stomach damage is alcohol [2]. By disrupting the equilibrium of the stomach mucosal barrier, stimulating the infiltration of inflammatory cells, and encouraging the release of inflammatory factors, ethanol harms the gastric mucosa [3, 4]. As is well known, GU is frequently characterized by stomach mucosal epithelial cell damage, which is a crucial component in its development. Numerous medications, including proton pump inhibitors, have been clinically utilized to treat GU [5, 6]. Omeprazole and other chemically created medications have significant negative effects and a high recurrence rate when used long-term to treat GU, however [6]. As a result, there is a need for a natural active ingredient that is both safe and efficient for treating and preventing ethanol-induced stomach damage. Researchers currently pay a lot of attention to using natural compounds like terpenoids, flavonoids, and polysaccharides derived from plant resources as medications or food supplements. In addition, Prior research has indicated that food additives, such as colorants, emulsifiers, preservatives, and stabilizers, which are extensively used in the food industry, can cause several toxic effects, including ulceration, alteration of gastric homeostasis, oxidative stress, cancers, and other adverse effects [2,3,4]. These agents induce inflammatory reactions in the gastric mucosa, leading to damage, alteration of the mucosa, and gastric cell necrosis [10, 11]. The mechanisms responsible for the lesion of the gastric mucosa are pro-inflammatory mediators, particularly the level of NO, which is a major defensive system of the gastric mucosa [12]. Currently, synthetic drugs used for ulcer treatment have toxic effects [13]. Therefore, the use of non-toxic natural medicines capable of preventing and treating gastric ulcers is one of the strategies for the treatment of gastric diseases and disturbances.

Functional food and natural food additives have the potential to become the future of the prevention of many chronic diseases, such as dyslipidemia, hyperglycemia, obesity, and cardiovascular diseases [14–17]. Betalains, which are red pigments found in several plants, such as red-prickly pear and beetroot, have been identified as a promising natural food additive. These pigments are widely used as food colorants in candy, creams, and juices [18–21]. Betanine (betanidin 5-O-β-D-glucoside, red) is the most abundant betalain component, accounting for approximately 90% of total betalains [10]. Several subsequent studies have reported that this compound possesses strong antioxidant and anti-inflammatory activities [22–26].Within this particular context, the primary objective of our study is to conduct an initial assessment of the impact of betanin consumption on the activity and gene expression of pivotal enzymes involved in gastric mucosal inflammation and ulceration.

2. Materials and Methods

Betanin extraction and chromatographic analysis. Fresh and fully matured beet roots were harvested in the month of June. These roots were manually washed and peeled, and a control sample (BV-Mo/16) was deposited at Laboratory LR11ES39 [27]. The total betalains were extracted by grinding the roots in a water/methanol/formic acid mixture (84.95/15/0.05) following the protocol described by Sawicki et al. [28]. Subsequently, the beetroot powder was freeze-dried, homogenized in a sonicated solvent mixture, and centrifuged for 10 minutes (13,000 x g at 4°C) for 20 minutes. Pure betanin was purified and characterized using UV-visible, HPLC-DAD-UV, and infrared spectroscopy (FTIR) in accordance with the protocol described by Gonçalves et al. [29]. The lyophilized betanin material was weighed and stored under refrigeration until its use.

In vitro anti-inflammatory assay. To ensure accurate and reliable measurements of enzyme activity in the present study, different protocols were utilized. For instance, the activity of 5-Lipoxygenase (sigma-437996-500U) was determined using the protocol established previously [30]. The method involved measuring the absorbance at 234 nm in a reaction medium consisting of a borate buffer mixture, the enzyme, and linoleic acid as substrate. Hyaluronidase activity in the gastric mucosa was determined using the protocol described by Nishida et al.[30]. which involved a mixture of the enzyme, BSA sodium phosphate buffer, hyaluronic acid, and albumin.

The activity of MPO was determined using the protocols described by Queiroz et al. and Almeida et al. [31, 32]. The reaction mixture consisted of MPO and samples (0.1, 0.2, 0.4, 0.8 and 1.6 mg/mL) in phosphate M buffer and the reaction was initiated by adding H2O2, catalase and 2-nitro-5-thiobenzoic acid. After incubation in the dark for 5 min, the absorbance was measured at 412 nm.

Docking betanin with the target Human enzymes: Human 5-lipoxygenase, hyaluronidase and myeloperoxidase structures were extracted from the RCSB PDB Protein Data Bank (pdb codes 6ncf, 2pe4 and 1d7w, respectively) (https://www.rcsb.org/). Before docking, water molecules were removed, and hydrogens were added. For the Lipoxygenase structure, the inhibitor (3-acetyl-11-keto-beta-boswellic acid) was removed. The Bet structure was generated using ChemBio3D Ultra 12.0 software (CambridgeSoft Co., USA) and its energy was minimized with the MM2 tools implemented to the software. The molecular docking of the Bet in the enzyme binding site was performed using autodock Vina software [64]. with a radius of 1.00. The docking box included the substrate binding cavity and had a size of 24. For the lipoxygenase the grid box included the catalytic and the inter-domain cavities. For the Lipoxygenase, docking box center coordinates were x: 8.798, y = 41.271 and z = 23.45. For hyaluronidase, the box was centered on catalytic Glu131, with center coordinates x: 37.461, y = -19.162 and z = -16.594. With regards to the myeloperoxidase, center coordinates were x: 20.836, y = -20.157 and z = 0.467. The best ranked docking pose of Bet compound in the active site of each enzyme was obtained according to the free binding-energy value. Structures were visualized, and figures were generated using the Biovia Studio visualizer (Accelrys

Software Inc., Discovery Studio Modeling Environment, Release 2017 R2, San Diego: Accelrys Software Inc., 2007).

Ethanol-induced gastric ulcer. Ulcer induction was done with absolute ethanol as described by Hafsa et al [33]. Male Wistar strain rats weighted of 181 ± 9 g and two months of age were kept under a controlled environment and a voluntary diet. Rats with gastric ulcer were grouped into 5 groups of 5 rats each.: group 1: formed of healthy rats (C); group 2 composed of rats with gastric ulcer induced by oral ethanol at a dose of 5 ml/kg body weight (bw) (U); group 3 composed of rats with gastric ulcer and treated with and omeprazole at a dose of 20 mg/kg daily; and groups 4 and 5 are ulcerated rats treated with purified betanin (at a dose of 30 and 60 mg/kg bw daily) The induction of ulcers was carried out using absolute ethanol, as previously described by Hafsa et al [28]. Male Wistar strain rats, weighing 181 ± 9 g and aged two months, were maintained under controlled environmental conditions, and provided with a voluntary diet. Rats with gastric ulcers were divided into five groups, each consisting of five rats: group 1 comprised healthy rats (C); group 2 comprised rats with gastric ulcers induced by oral ethanol at a dose of 5 ml/kg bw (U); group 3 comprised rats with gastric ulcers treated with omeprazole at a dose of 20 mg/kg daily; and groups 4 and 5 comprised ulcerated rats treated with purified betanin at doses of 30 and 60 mg/kg bw daily, respectively. Following sacrifice, the stomachs of the rats were gently washed with ice-cold phosphate buffer. The experimental procedures involving rats were conducted in accordance with the recommendations of the National Ethics Committee (approval number: CEEA-ENMV 23/20) and the animal care and use commission (approval number: CER-SVS 0013/20222020-0205), in compliance with the U.S. National Institutes of Health's "Guide for the Care and Use of Laboratory Animals" (NIH Publication No. 85-23, revised 1996). Three hours after the administration of absolute ethanol, the animals were sacrificed, and their stomachs were quickly removed, cleaned with physiological water, and the surface area of the gastric ulcer was determined using an inverted microscope associated with a digital camera. The treatments of the gastric surface were analyzed using the Image J software with a calculable numerical distance (mm). The gastric mucosa content was estimated using the protocol of Ofusori et al. [34].The ulcer index (UI) and healing index (CI) were calculated according to the method described in this study [35].

Biochemical analysis. The present study involved the determination of various biochemical parameters in gastric juice. Specifically, the levels of TBARS were calculated using the methodology outlined by Buege and Aust [36]. The gastric NO rate was determined using the protocol described by [37]. The protein level was calculated in accordance with the instructions provided by the manufacturer (Kit biolabo, France, ref K2016). The hyaluronidase activity of gastric juice was assessed by measuring the rate of n-acetylglucosamine formation from hyaluronate via absorbance [38]. Finally, the MPO activity was determined using the methodology described by Hillegas et al. [31].

RNA extraction and semi-quantitative RT-PCR. The guanidium thiocyanate-phenol-chloroform method, as described by Hamden et al [39, 40], was utilized to extract total gastric mucosal RNAs. In brief, the gastric mucosa of rats was homogenized in lysis buffer [39,40], followed by incubation in cold and subsequent centrifugation. The RNAs were then precipitated at –80°C with isopropanol, washed, and stored at –80°C until use. The quantity of total RNA was determined by measuring the absorbance at 260 nm, while the purity was assessed by measuring the 260/280 nm ratio. The quality of the RNA was evaluated by migration on 1.5% agarose gel. Subsequently, total RNAs were reverse transcribed into cDNA by RT-PCR. The cDNAs of the target genes and of L19 were amplified by PCR using specific primers (Table 1) as indicated elsewhere. The cDNAs of the target genes were then migrated on a 2% agarose gel stained with ethidium bromide, visualized under UV transillumination, and analyzed using NIH software (http://rsb.info.nih.gov/nih-image).

Table 1. The primer sequences used for RT-PCR.

Gene name	Forward primer (5'– 3')	Reverse primer (5'–3')
TNFα	GCAGGTCTACTTTGGGATCATT	AGAAGAGGTTGAGGGTGTCT
iNOS	CAGCACAGAGGGCTCAAAGG	TCGTCGGCCAGCTCTTTCT
COX2	CCAAACCAGCAGGCTCATACT	AGCGGATGCCAGTGATAGAGT
NF-ϰB	AGTTGAGGGGACTTTCCCAGGC	TCAACTCCCCTGAAAGGGTCCG
L19	GAAATCGCCAATGCCAACTC	ACCTTCAGGTACAGGCTGTG

Statistical Analysis. The present study was conducted using a sample size of five rats per group. The statistical analysis of the data was performed using the Fisher test and one-way analysis of variance (ANOVA) through the Stat View software. The variations in the values were determined based on statistical analysis. The statistical significance was considered at a level of $p \leq 0.05$.

In case you need to itemize parts of your text, use either bullets or numbers.

3. Results

Assessment of Bet compound effect on pivotal enzymes associated with inflammation.

In Vitro analysis. The present study conducted in vitro has demonstrated that Bet exhibits a potent and dose-dependent inhibitory effect on the crucial enzymes involved in gastric inflammation, namely 5-lipoxygenase (5-LO), hyaluronidase (HAase), and myeloperoxidase (MPO) (**Fig. 1**). The inhibitory concentration (IC50) of Bet against 5-LO, HAase, and MPO was found to be 79.7, 101.4, and 31.4 µg/ml, respectively.

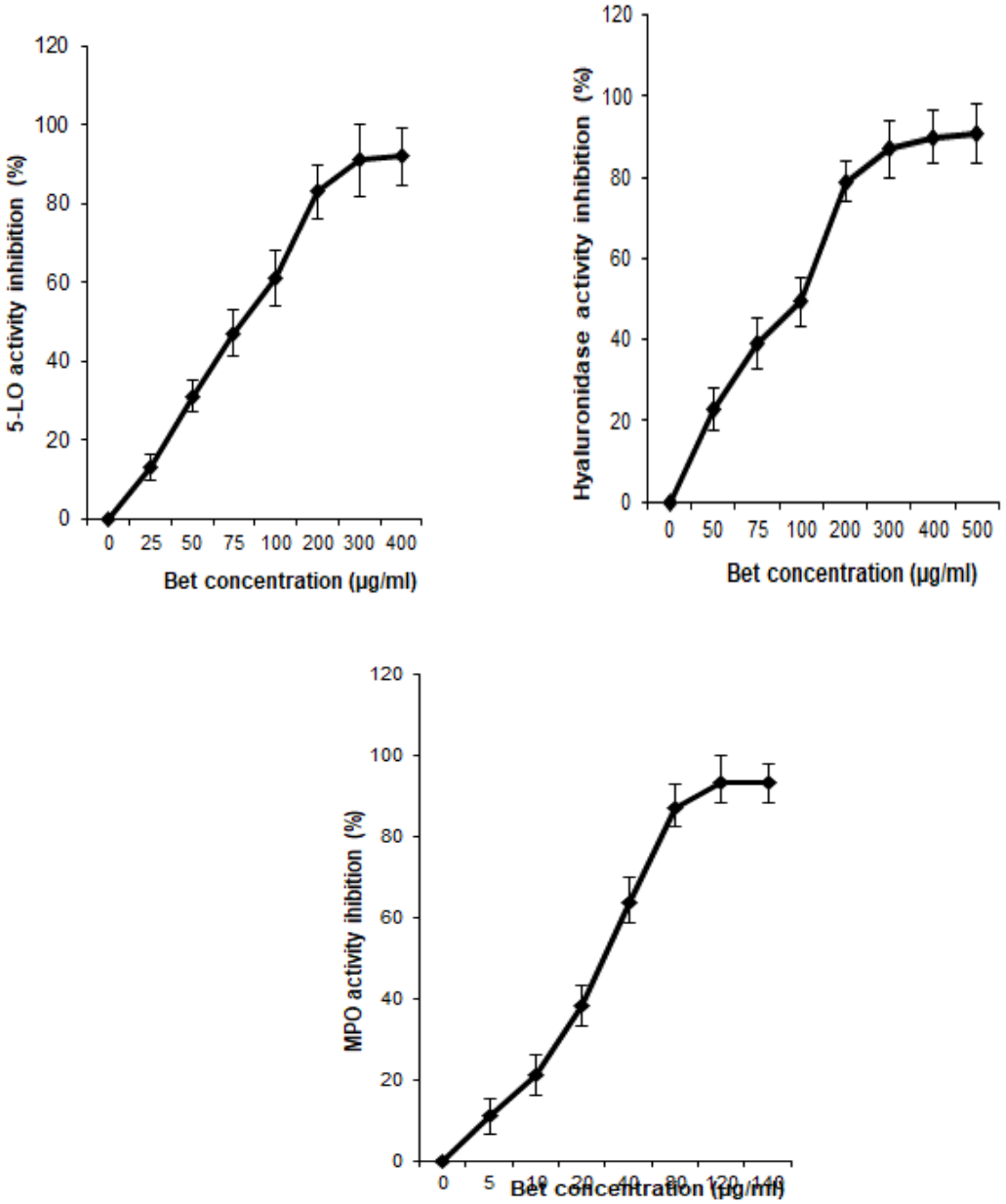

Figure 1. In vitro assessment of the inhibitory effect of betanin on the activities of key enzymes involved in gastric inflammation: 5-Lipoxygenase, Hyaluronidase, and Myeloperoxidase.

Relationship between bet ingestion and key enzymes associated with inflammation in the gastric mucosa of ulcerated rats. The findings of our study demonstrate that the administration of

absolute ethanol at a dosage of 5ml/kg bw induced pronounced inflammation in the gastric mucosa, as evidenced by a substantial elevation in 5-LO, HAase, and MPO activities by 179%, 130%, and 106% respectively (**Fig. 2**).

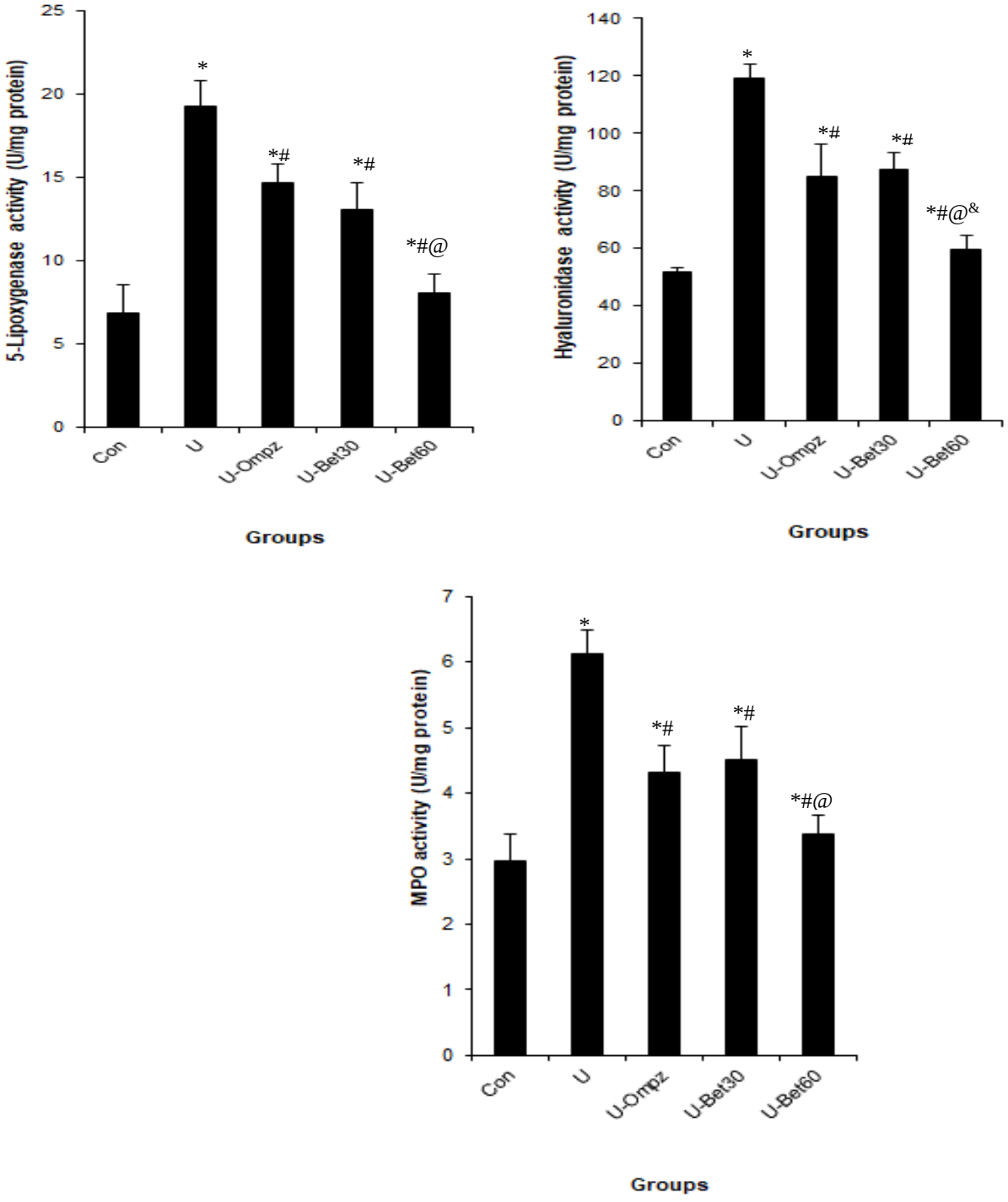

Figure 2. Effect of Bet treatment on the activities of the gastric mucosa 5-Lipoxygenase, Hyaluronidase, and Myeloperoxidase. Statistical analysis as *P < 0.05 significant differences compared to controls; #P < 0.05 significant differences compared to U; $^{@}$P < 0.05 significant differences compared to U-Ompz; $^{\&}$P < 0.05 significant differences compared to U-Bet$_{30}$.

Conversely, in ulcerated rats subjected to oral administration of Bet, the gastric mucosa exhibited a safeguarding effect against inflammation. In fact, the consumption of Bet by ulcerated rats significantly inhibited 5-LO, HAase, and MPO activities by 54%, 49%, and 45% respectively.

Docking of betanin with the target enzymes. To understand the inhibitory effect of betanin towards the target Human enzymes, docking studies were carried out. For the hyaluronidase, the binding energy of betanin was -8.4 Kcal/mol. The betanin is located with the enzyme active site close to key catalytic residues Asp129, Glu131 and Tyr247 (**Fig. 3A** and **3B**). The inhibitor is stabilized in the active site mainly by hydrogen bonding with residues Glu131, Asp292, Val322 and Trp324 (**Fig. 3B** and **3C**).

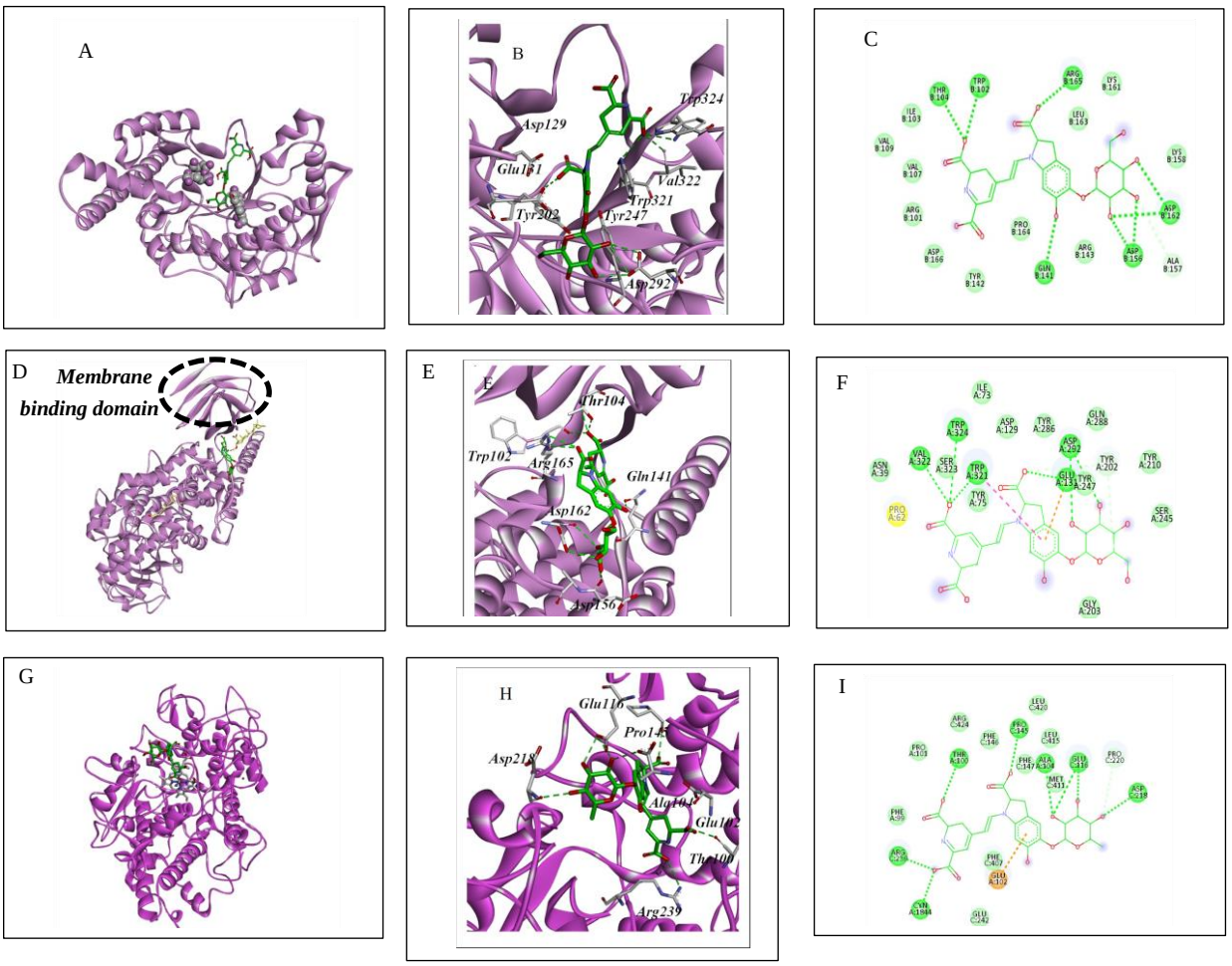

Figure 3. Hyaluronidase (A, B and C), Lipoxygenase (D, E and F), and myeloperoxidase (G, H and I) in complex with betanin. Left panels (A, D and G): Ribbon representation of the enzyme (Lipoxygenase, hyaluronidase or myeloperoxidase) in complex with the betanin (green sticks). Two Inhibitors described to bind to the catalytic site of lipoxygenase (A) and close to the membrane binding domain are shown as yellow sticks. In the case of Myeloperoxidase (G) the heme group (white sticks) located in the active site of the enzyme is shown. Middle panels (B, E and H), residues of the active

site interacting with betanin. Interacting residues are shown in white sticks. Hydrogen bonds are indicated by green dashed line. Right panels (C, F and I): 2D-diagrams of the betanin bound to the active site of the enzymes.

This binding mechanism is in line with the inhibitory effect of betanin towards the hyaluronidase.

The best ranked pose for betanin in complex with 5-lipoxygenase (Binding energy -7.1 Kcal/mol) is located between the catalytic and the membrane binding domain (**Fig. 3D** and **3E**). This binding site is close to that of the natural compound inhibitor 3-acetyl-11-keto-beta-boswellic acid (**Fig. 3D**), located between membrane binding and catalytic domains. Residues Trp102, Thr104, Gln141, Asp156, Asp162, and Arg165 are establishing hydrogen bonds with the inhibitor (**Fig. 3E** and **3F**).

The binding energy for the Bet to myeloperoxidase was -8.5 Kcal/mol. The binding pose was located within the enzyme catalytic site close to the catalytic heme group taking part in the oxidoreductase activity (**Fig. 3G** and **3H**). The ligand was stabilizing in the active site through hydrogen bonding with residues Thr100, Glu102, Ala104, Glu116, Pro145, Asp218 and Arg229 (**Fig. 3H** and **3I**).

Bet ingestion, TBARS and NO levels in gastric mucosa of ulcerated rats. The results of this study demonstrate that the administration of Bet in rats with ulcers effectively prevents and safeguards against cellular damage to the gastric mucosa. This is evidenced by a noteworthy decrease in the level of TBARS by 59% and a substantial increase in the level of gastric NO by 107% when compared to ulcerated rats that did not receive anything (**Fig. 4**).

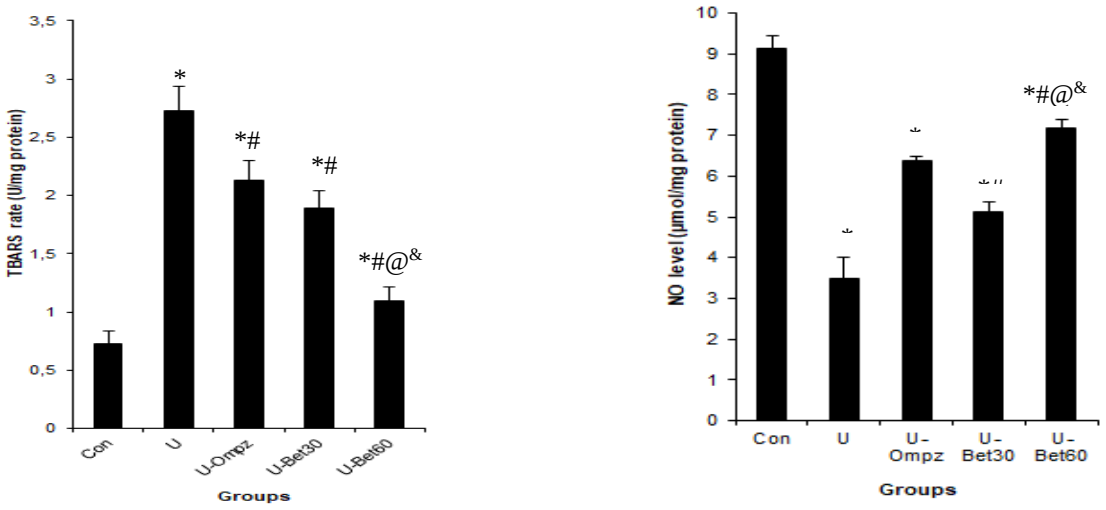

Figure 4. Effect of ingestion of the Beast by ulcerated rats on the level of TBARS and NO in the gastric mucosa. The results of this study show that the administration of Bet in ulcerated rats protects gastric cells against damage by suppressing the rate of lipid peroxidation and inducing defensive antioxidant power by stimulating NO synthesis. Statistical analysis as *P < 0.05 significant differences compared to controls; #P < 0.05 significant differences compared to U; $^{@}$P < 0.05 significant differences compared to U-Ompz; $^{\&}$P < 0.05 significant differences compared to U-Bet$_{30}$.

The impact of Bet and ulcer on genes expression (NF-ϰB, iNOS, COX-2 and TNF-α) of mRNA in gastric mucosa. This study aims to investigate the influence of bet and ulcer on the expression of nuclear factor kappa B (NF-ϰB), inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), and tumor necrosis factor-alpha (TNF-α) mRNA in the gastric mucosa. The examination of these molecular markers is crucial in understanding the underlying mechanisms involved in the pathogenesis of gastric ulcers.

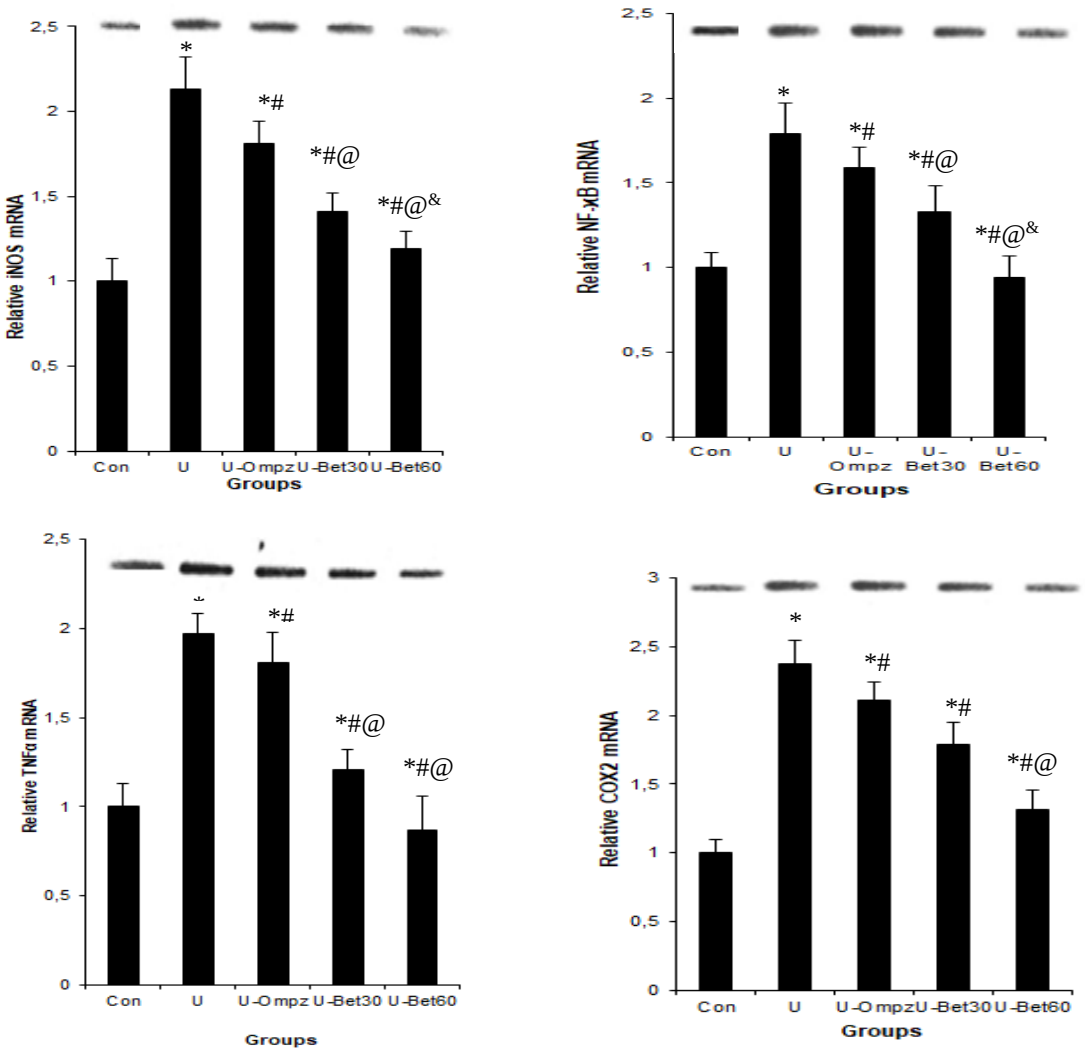

Figure 5. Effect of ulcer and Bet ingestion on the mRNA level of key genes propagating inflammation in gastric tissues such as NF-ϰB, iNOS, COX-2 and TNF-α. This study clearly shows the potential anti-inflammatory effect of Bet through the down-regulation of the expression of these genes. Statistical analysis as *P < 0.05 significant differences compared to controls; #P < 0.05 significant differences compared to U; $^{@}$P < 0.05 significant differences compared to U-Ompz; $^{\&}$P < 0.05 significant differences compared to U-Bet$_{30}$.

By analysing the mRNA expression levels of NF-ϰB, iNOS, COX-2, and TNF-α, this research aims to provide valuable insights into the molecular alterations occurring in the gastric mucosa during ulcer development and the potential role of bet in modulating these changes. The results of this study have demonstrated that the consumption of ethanol leads to a significant upregulation of the key inflammatory gene, NF-ϰB, by 79% (**Fig. 5**).

This upregulation of NF-ϰB mRNA levels subsequently triggers a cascade of gene induction responsible for inflammatory reactions, including iNOS, COX-2, and TNF-α, which were found to be upregulated by 113%, 137%, and 78%, respectively (**Fig. 5**). However, the administration of Bet via gavage was found to confer protection against inflammation of the gastric mucosa, as evidenced by the suppression of NF-ϰB mRNA synthesis by 47.4%. This downregulation of NF-ϰB mRNA levels was accompanied by a reduction in the expression of genes responsible for gastric cell damage, namely iNOS, COX-2, and TNF-α, which were found to be downregulated by 44%, 45%, and 56%, respectively, when compared to untreated ulcerated rats. This finding may contribute to the development of novel therapeutic strategies for the management of gastric disorders.

The effects of Bet ulcerated treatment on gastric macroscopic injury, gastric mucosa content, ulcer area, and curative index in rats. The present study shows that the administration of absolute ethanol in rats results in severe gastric bleeding and gastric mucosal ulceration (**Fig. 6**).

This damage to the gastric mucosa is evidenced by an increase in the surface area of the ulcerated mucosa and gastric secretion by 300% and 161%, respectively, when compared to healthy rats. Additionally, gastric ulceration leads to a reduction in the weight of the gastric mucosa by 59.7% (**Fig. 6**). However, treatment of ulcerated rats with Bet at a dose of 60mg/kg bw resulted in significant protection of the gastric mucosa. Specifically, bet ingestion reduced the surface area of ulcerated mucosa and gastric secretion by 75% and 40%, respectively, when compared to untreated ulcerated rats. Furthermore, Bet administration significantly increased the weight of the gastric mucosa by 117%, resulting in a curative index of 74% (**Fig. 6**).

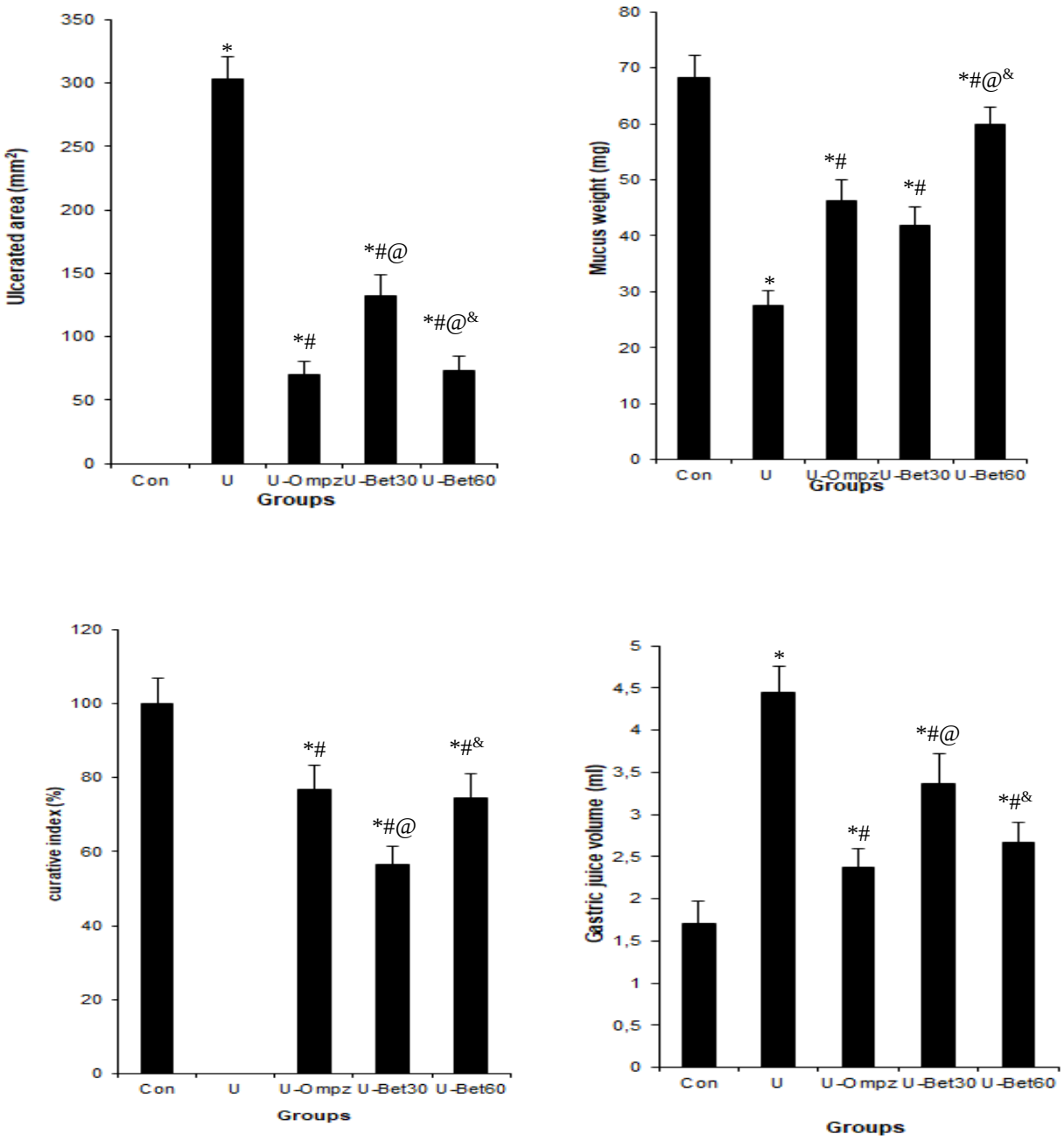

Figure 6. Gastric macroscopic injury, gastric mucosa content, ulcer area and curative index in Bet ulcerated treated. A potential protective effect was observed by Bet against gastric ulcer indices. Statistical analysis as *P < 0.05 significant differences compared to controls; #P < 0.05 significant differences compared to U; $^{@}$P < 0.05 significant differences compared to U-Ompz; $^{\&}$P < 0.05 significant differences compared to U-Bet_{30}.

4. Discussion

Gastric ulcer (GU) is a painful gastric disease that significantly affects the quality of life of many of patients. According to recent studies, the prevalence of GU ranges from 20 to 60 cases per 100,000 individuals , and it is responsible for nearly10% of global mortality [41, 42].

Gastric ulcer is a painful lesion of the gastric mucosa that arises due to inflammatory reactions, which can result in stomach perforation and internal bleeding [11]. Clinical management of ulcerative gastritis (UG) involves the use of medications with various mechanisms, including antisecretory gastric acid agents such as H2 receptor antagonists (e.g., cimetidine) and proton pump inhibitors (e.g., omeprazole), antibiotics, antacids, and mucosal protective agents [43–45]. However, the use of these drugs is associated with the problem of UG recurrence, undesirable side effects, and the inability to address the underlying cause of this condition, which is the inflammatory reactions in the gastric mucosa. Therefore, the exploration of food compounds, particularly natural food additives such as dyes, is an alternative approach to eliminate toxic synthetic food additives, promote healthy food, and prevent various health disorders [6, 46–50]. It is well-established that ethanol ingestion induces inflammatory reactions in the gastric mucosa, as evidenced by the induction of inflammatory genes and enzymes, epithelial cell necrosis, and increased gastric permeability [6, 46–50].The present study demonstrated that the consumption of ethanol leads to the degradation of purines, resulting in an excessive production of reactive oxygen species (ROS), which are known to mediate oxidative stress. This oxidative stress triggers the migration of neutrophils towards the damaged area of the gastric tissue. Subsequently, the neutrophils aggregate in the gastric mucosa, leading to the formation of lesions and inflammation due to the release of tissue-destructive substances such as proteases and leukotrienes [51]. Our study specifically identified the induction of 5-lipoxygenase (5-LO), hyaluronidase (HAase), and myeloperoxidase (MPO) activities because of this oxidative stress in the gastric mucosa. This was further supported by a significant increase in thiobarbituric acid reactive substances (TBARS) levels and a decrease in nitric oxide (NO) content.

In contrast, the consumption of Bet exhibited a down-regulatory effect on the activity of key enzymes associated with oxidative stress. This was evidenced by a significant reduction in the activity of 5-LO, HAase, and MPO, an increase in NO content, and a decrease in TBARS levels, indicating a suppression of gastric cell damage. These findings are consistent with previous literature [52–55]. Additionally, previous studies have reported that a decrease in the activity of inflammatory enzymes, namely 5-LO, HAase, and MPO, as well as a reduction in TBARS levels, serve as indicators of decreased leukocyte influx in the gastric mucosa. This reduction is associated with a decrease in histopathological alterations, gastric haemorrhage, and exhibits anti-ulcer activity. The strong stabilization of ligand in

the entry of the active site suggested an inhibitor site for bet in line with its high inhibitory power towards the enzymes.

Bet binding with the catalytic cavity for the hyaluronidase and myeloperoxidase can explain its inhibitory power of both enzymes. In the case of the lipoxygenase, Bet is binding between the catalytic and the membrane binding domain. This binding site was described for natural compounds impairing the lipoxygenase activity such as 3-acetyl-11-keto-beta-boswellic acid Structural and mechanistic insights into 5-lipoxygenase inhibition by natural products [65]. Binding to this allosteric site located at 40 Å from the catalytic binding site was proposed as a new mechanism of enzymatic activity control. In addition, the administration of Bet in ulcerated rats leads to a down-regulation of the key gene involved in inducing inflammation in the gastric mucosa, specifically NF- ϰb. This down-regulation is evident through a significant suppression of the mRNA level of NF-ϰB [56].The inhibition of NF-ϰB gene expression and consequently its protein level in the gastric mucosa inhibits the cascade of inflammatory reactions by suppressing the expression of genes involved in the inflammatory cascade, namely iNOS, TNFα and COX2 [57]. Our findings show that the ingestion of Bet by ulcerated rats results in a suppression of gastric mucosa iNOS, TNF-α and COX-2 mRNA levels by 113, 78 and 137%, respectively compared to untreated rats with ulcers. These results are in agreement withZheng et al [57], who reported that the ingestion of ethanol induces inflammatory reactions by stimulating the secretion of pro-inflammatory cytokines such as TNF-α, IL-6, IFN)-c, IL-1β, while reducing anti-inflammatory cytokines like IL-10. Additionally, Zheng et al demonstrated that the administration of natural compounds such as costunolide and dehydrocostuslactone can reduce these inflammatory cytokines by suppressing the expression of the NF-ϰB gene, thereby protecting gastric tissues from ulceration. Other studies [51, 58, 59] have also shown that the induction of NF-κB promotes ulceration and cell damage in the gastric mucosa, while the suppression of this gene promotes the repair of gastric tissue damage and ulceration. Previous studies have documented the significant role of nitric oxide (NO) in the defence against inflammation [60]. Our findings indicate that the administration of Bet in ulcerated rats leads to a substantial increase in gastric NO levels. This increase can be attributed to the potent anti-inflammatory activity of Bet. Furthermore, maintaining high levels of NO plays a crucial role in protecting the gastric mucosa [61]. Our results align with Liang et al , [61] who observed that the gastroprotective effects of anti-ulcer compounds are likely mediated by the induction of NO levels as a defence mechanism against damage and inflammation. Another study by Salem et al [50] demonstrated that the gastroprotective effect of Cornu aspersum mucin is attributed to its ability to reduce the expression of iNOS and induce NO levels.

In the present study, we have demonstrated that Bet inhibits oxidative stress and suppresses lymphocyte migration and infiltration in the intestinal mucosa. This leads to a reduction in reactive oxygen species (ROS) production and is accompanied by a direct interaction and inhibition of enzymes, as previously shown in vitro. Notably, bet possesses various functional properties, making it a natural food colouring agent for several food products such as yogurts, ice creams, jams, chewing gums, sauces, and soups. It

is characterized by its potent capacity to neutralize free radicals and its ability to induce the expression of genes encoding antioxidant and detoxification enzymes, including kinase pathways such as AKT, JNK, and ERK [62]. Conversely, this pigment inhibits the genes responsible for the cytokines involved in the inflammatory reaction cascade, such as Toll-like receptor 4 and NF-κB [63]. The present study provides clear evidence that the consumption of ethanol leads to the development of oxidative stress and excessive production of reactive oxygen species (ROS) in gastric tissues. This stress triggers the migration of neutrophils to the gastric mucosa, which in turn initiates a series of inflammatory reactions in the mucosa. These reactions are mediated by the activation of specific target genes, such as NF-ϰB, and the inhibition of anti-inflammatory genes, including those responsible to produce nitric oxide (NO). Consequently, these inflammatory reactions result in the infiltration of lymphocytes and severe damage to the gastric mucosa, characterized by extensive haemorrhage and degradation, ultimately leading to the formation of ulcers, as shown in the figure 6.

However, in rats with ulceration that were treated with Bet, a strong anti-ulcer effect was observed. Our findings clearly demonstrate that the consumption of Bet reduces oxidative stress in gastric tissues, as evidenced by a significant decrease in the activity of key enzymes involved in inflammation, such as 5-lipoxygenase (5-LO), hyaluronidase (HAase), and myeloperoxidase (MPO). Additionally, there was a notable suppression of the rate of thiobarbituric acid reactive substances (TBARS), which is indicative of reduced lipid peroxidation. This reduction in oxidative stress resulted in a down-regulation of ROS production. Consequently, the lower levels of ROS recruited a relatively smaller number of neutrophils to the gastric mucosa. This suppression of lymphocytic infiltration following the consumption of Bet significantly mitigated the inflammatory reactions, thereby preventing substantial alterations to the mucosa and exhibiting a considerable anti-ulcer effect.

5. Conclusions

This study presents evidence that the administration of ethanol leads to the development of gastric ulceration by inducing the excessive production of reactive oxygen species (ROS). This, in turn, triggers a significant infiltration of lymphocytes within the gastric mucosa, resulting in a cascade of inflammatory responses. Conversely, the administration of Bet effectively reduces ROS levels, thereby mitigating lymphocyte infiltration and potentially suppressing inflammatory reactions by down-regulating inflammation-associated target genes within gastric tissues. These results suggest that Bet exhibits significant anti-ulcer properties, thereby potentially offering valuable insights for the advancement of innovative therapeutic approaches in the treatment of gastric ailments.

Compliance with Ethical Standards

The experiment was conducted in accordance with the National Ethics Committee (approval number: CEEA-ENMV 23/20) and the animal care and use commission (approval number: CER-SVS

0013/20222020-0205), in compliance with the U.S. National Institutes of Health's "Guide for the Care and Use of Laboratory Animals" (NIH Publication No. 85-23, revised 1996), date of approval Jenuary 4, 2022)" for animal studies.

Conflict of Interest

The author states no conflict of interest with respect to the research, authorship, and/or publication of this article.

References

1. Hassan H.M. et al. 2023. Genistein ameliorated experimentally induced gastric ulcer in rats via inhibiting gastric tissues fibrosis by modulating Wnt/β-catenin/TGF-β/PKB pathway. Redox Rep. **28**, 2218679.
2. Shin M.-S., Lee J., Lee J.W., Park S.H., Lee I.K., Choi J.A., Lee J.S., Kang K.S. 2021. Anti-inflammatory effect of Artemisia argyi on ethanol-induced gastric ulcer: analytical, in vitro and in vivo studies for the identification of action mechanism and active compounds. Plants. **10**, 332.
3. Lin L., Xie B., Shi J., Zhou C.M., Yi J., Chen J., He J.X., Wei H.L. 2023. IL-8 Links NF-κB and Wnt/β-Catenin Pathways in Persistent Inflammatory Response Induced by Chronic Helicobacter pylori Infection. Mol. Biol. 1–12.
4. Birerdinc A., Mehta R., Alhussain R., Afendi A., Chandhoke V., Younossi Z., Baranova A. 2012. Selection of reliable reference genes for qRT-PCR analysis in human non-cancerous gastric tissue. Mol. Biol. **46**, 153–60.
5. Liu X., Quan S., Han Q., Li J., Gao X., Zhang J., Liu D. 2022. Effectiveness of the fruit of Rosa odorata sweet var. gigantea (Coll. et Hemsl.) Rehd. et Wils in the protection and the healing of ethanol-induced rat gastric mucosa ulcer based on Nrf2/NF-κB pathway regulation. J. Ethnopharmacol. **282**, 114626.
6. Zhou C., Chen J., Liu K., Maharajan K., Zhang Y., Hou L., Li J., Mi M., Xia Q. 2023. Isoalantolactone protects against ethanol-induced gastric ulcer via alleviating inflammation through regulation of PI3K-Akt signaling pathway and Th17 cell differentiation. Biomed. Pharmacother. **160**, 114315.
7. Tarlo S.M., Sussman G.L. 1993. Asthma and anaphylactoid reactions to food additives. Can.

Fam. Physician. **39**, 1119.

8. Bhattacharyya S. et al. 2017. A randomized trial of the effects of the no-carrageenan diet on ulcerative colitis disease activity. Nutr. Heal. aging. **4**, 181–92.
9. Choi H.J., Kim J., Park S.-H., Do K.H., Yang H., Moon Y. 2012. Pro-inflammatory NF-κB and early growth response gene 1 regulate epithelial barrier disruption by food additive carrageenan in human intestinal epithelial cells. Toxicol. Lett. **211**, 289–95.
10. Saad F., Al-Shaikh T.M., Zouidi F., Taher M.A., Saidi S.A., Hamden K. 2023. Betalain-Enriched Beetroots Exhibit Antiulcer and Anti-inflammatory Potentials. Ed A. Zeb. J. Food Process. Preserv. **2023**, 9522830.
11. Abdelkader Saidi S., Al-Shaikh T.M., Hamden K. 2023. Evaluation of Gastroprotective Effect of Betalain-Rich Ethanol Extract from Opuntia stricta var. Dillenii Employing an In Vivo Rat Model. J. Food Qual. **2023**.
12. Dambrova M., Zvejniece L., Skapare E., Vilskersts R., Svalbe B., Baumane L., Muceniece R., Liepinsh E. 2010. The anti-inflammatory and antinociceptive effects of NF-κB inhibitory guanidine derivative ME10092. Int. Immunopharmacol. **10**, 455–60.
13. de Lira Mota K.S., Dias G.E.N., Pinto M.E.F., Luiz-Ferreira Â., Monteiro Souza-Brito A.R., Hiruma-Lima C.A., Barbosa-Filho J.M., Batista L.M. 2009. Flavonoids with gastroprotective activity. Molecules. **14**, 979–1012.
14. Saidi S.A., Al-Shikh T.M., Hamden K. 2022. Ephedra alata subsp. alenda (Ephedraceae) leaf extracts: phytochemical screening, anti-diabetic, anti-obesity and anti-toxic activities on diabetic-induced liver-kidney-testes toxicities and inhibition of α-amylase and lipase enzymes. Heliyon. e11954.
15. Tiss M., Souiy Z., Abdeljelil N. ben, Njima M., Achour L., Hamden K. 2020. Fermented soy milk prepared using kefir grains prevents and ameliorates obesity, type 2 diabetes, hyperlipidemia and Liver-Kidney toxicities in HFFD-rats. J. Funct. Foods. **67**, 103869.
16. Hamden K., Boujibiha M.A., Ben Abdeljelil N., Njima M., Selmi B., Achour L. 2019. Phytoestrogens inhibit key-enzymes linked to obesity, type 2 diabetes and liver-kidney toxicity in high fructose-fat diet in mice. Arch. Physiol. Biochem. **125**, 423–9.

17. Hamden K., Jaouadi B., Carreau S., Aouidet A., El-Fazaa S., Gharbi N., Elfeki A. 2010. Potential protective effect on key steroidogenesis and metabolic enzymes and sperm abnormalities by fenugreek steroids in testis and epididymis of surviving diabetic rats. Arch. Physiol. Biochem.

18. Roriz C.L. et al. 2022. Red pitaya (Hylocereus costaricensis) peel as a source of valuable molecules: Extraction optimization to recover natural colouring agents. Food Chem. **372**, 131344.

19. Singh T., Pandey V.K., Dash K.K., Zanwar S., Singh R. 2023. Natural bio-colorant and pigments: Sources and applications in food processing. J. Agric. Food Res. **12**, 100628.

20. Dhiman A., Suhag R., Chauhan D.S., Thakur D., Chhikara S., Prabhakar P.K. 2021. Status of beetroot processing and processed products: Thermal and emerging technologies intervention. Trends Food Sci. Technol. **114**, 443–58.

21. Prajapati R.A., Jadeja G.C. 2022. Natural food colorants: Extraction and stability study. Mater. Today Proc. **57**, 2381–95.

22. Shafqat O., Rehman Z., Shah M.M., Ali S.H.B., Jabeen Z., Rehman S. 2023. Synthesis, structural characterization and in vitro pharmacological properties of betanin-encapsulated chitosan nanoparticles. Chem. Biol. Interact. **370**, 110291.

23. Wang B., Wang Y., Deng Y., Yao Q., Xiong A. 2023. Synthesis of betanin by expression of the core betalain biosynthetic pathway in carrot (Daucus carota L.). Hortic. Plant J.

24. Xu J., Lai H., You L., Zhao Z. 2022. Improvement of the stability and anti-AGEs ability of betanin through its encapsulation by chitosan-TPP coated quaternary ammonium-functionalized mesoporous silica nanoparticles. Int. J. Biol. Macromol. **222**, 1388–99.

25. de Freitas Marinho L., Sganzerla W.G., Ferreira V.C., Jimenez Moreno J.A., Rostagno M.A., Forster-Carneiro T. 2023. Advances in green extraction methods, biological properties, and applications of betanin and vitexin: An updated review and bibliometric analysis. Biocatal. Agric. Biotechnol. **51**, 102744.

26. Wu Z., Chen L., Wang Q., Govindasamy C., Subramaniyan Sivakumar A., Chen X. 2023. Betanin ameliorates Lipopolysaccharide-induced acute lung injury in mice via inhibition of

inflammatory response and oxidative stress. Arab. J. Chem. **16**, 104763.

27. Zardi-Bergaoui A., Ben Nejma A., Harzallah-Skhiri F., Flamini G., Ascrizzi R., Ben Jannet H. 2017. Chemical composition and biological studies of the essential oil from aerial parts of Beta vulgaris subsp. maritima (L.) arcang. growing in Tunisia. Chem. Biodivers. **14**, e1700234.
28. Sawicki T., Bączek N., Wiczkowski W. 2016. Betalain profile, content and antioxidant capacity of red beetroot dependent on the genotype and root part. J. Funct. Foods. **27**, 249–61.
29. Gonçalves L.C.P., de Souza Trassi M.A., Lopes N.B., Dörr F.A., dos Santos M.T., Baader W.J., Oliveira Jr V.X., Bastos E.L. 2012. A comparative study of the purification of betanin. Food Chem. **131**, 231–8.
30. Nishida Y., Sugahara S., Wada K., Toyohisa D., Tanaka T., Ono M., Yasuda S. 2014. Inhibitory effects of the ethyl acetate extract from bulbs of Scilla scilloides on lipoxygenase and hyaluronidase activities. Pharm. Biol. **52**, 1351–7.
31. Queiroz R.F., Vaz S.M., Augusto O. 2011. Inhibition of the chlorinating activity of myeloperoxidase by tempol: revisiting the kinetics and mechanisms. Biochem. J. **439**, 423–34.
32. Almeida V.M., Dias Ê.R., Souza B.C., Cruz J.N., Santos C.B.R., Leite F.H.A., Queiroz R.F., Branco A. 2022. Methoxylated flavonols from Vellozia dasypus Seub ethyl acetate active myeloperoxidase extract: in vitro and in silico assays. J. Biomol. Struct. Dyn. **40**, 7574–83.
33. Izhar H., Shabbir A., Shahzad M., Mobashar A., Ahmed S.S. 2021. *Phyllanthus reticulatus* Prevents Ethanol-Induced Gastric Ulcer via Downregulation of IL-8 and TNF-α Levels. Ed S. Smaoui. Evidence-Based Complement. Altern. Med. **2021**, 1734752.
34. Ofusori A.E., Moodley R., Jonnalagadda S.B. 2020. Antiulcerogenic effects of Celosia trigyna plant extracts on ethanol-induced gastric ulcer in adult Wistar rats. J. Tradit. Complement. Med. **10**, 586–93.
35. Saad F., Al-Shaikh T.M., Zouidi F., Taher M.A., Saidi S.A., Hamden K. 2023. Betalain-Enriched Beetroots Exhibit Antiulcer and Anti-inflammatory Potentials. Ed A. Zeb. J. Food Process. Preserv. **2023**, 9522830.
36. Buege J.A., Aust S.D. 1978. [30] Microsomal lipid peroxidation. In: Methods in enzymology., vol. 52. Elsevier, pp. 302–10.

37. Miranda K.M., Espey M.G., Wink D.A. 2001. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric oxide. **5**, 62–71.

38. Abhijit S., Manjushree D. 2010. Anti-hyaluronidase, anti-elastase activity of Garcinia indica. Int. J. Bot. **6**, 299–303.

39. Hamden K., Silandre D., Delalande C., ElFeki A., Carreau S. 2008. Protective effects of estrogens and caloric restriction during aging on various rat testis parameters. Asian J. Androl.

40. Hamden K., Silandre D., Delalande C., Feki A. El, Carreau S. 2008. Age-related decrease in aromatase and estrogen receptor (ERα and ERβ) expression in rat testes: protective effect of low caloric diets*. Asian J AndrolAsian J Androl. **10**, 177–87.

41. Sung J.J.Y., Tsoi K.K.F., Ma T.K.W., Yung M.-Y., Lau J.Y.W., Chiu P.W.Y. 2010. Causes of mortality in patients with peptic ulcer bleeding: a prospective cohort study of 10,428 cases. Off. J. Am. Coll. Gastroenterol. ACG. **105**, 84–9.

42. Ren S. et al. 2020. Rutaecarpine ameliorates ethanol-induced gastric mucosal injury in mice by modulating genes related to inflammation, oxidative stress and apoptosis. Front. Pharmacol. **11**, 600295.

43. Vecchia C.A.D. et al. 2022. Sonchus oleraceus L. Promotes gastroprotection in rodents via antioxidant, anti-inflammatory, and antisecretory activities. Evidence-Based Complement. Altern. Med. **2022**.

44. Pleuvry B.J. 2009. Gastric disorders: modifications of gastric content, antacids and drugs influencing gastric secretions and motility. Anaesth. Intensive Care Med. **10**, 351–4.

45. Garner A. 1992. Adaptation in the pharmaceutical industry, with particular reference to gastrointestinal drugs and diseases. Scand. J. Gastroenterol. **27**, 83–9.

46. Aydın M.S., Eren M.A., Uyar N., Kankılıç N., Karaaslan H., Sabuncu T., Çelik H. 2023. Relationship between systemic immune inflammation index and amputation in patients with diabetic foot ulcer. J. Orthop. Sci.

47. Selim H.M., Negm W.A., Hawwal M.F., Hussein I.A., Elekhnawy E., Ulber R., Zayed A. 2023. Fucoidan mitigates gastric ulcer injury through managing inflammation, oxidative stress, and NLRP3-mediated pyroptosis. Int. Immunopharmacol. **120**, 110335.

48. Chetty T., Nowak B.F., Walker S.P., Symonds J.E., Anderson K. 2023. Molecular evidence for stress, inflammation and structural changes in non-specific ulcers in skin of farmed Chinook salmon (Oncorhynchus tshawytscha). Fish Shellfish Immunol. **137**, 108739.

49. Ma N., Sun Y., Yi J., Zhou L., Cai S. 2022. Chinese sumac (Rhus chinensis Mill.) fruits alleviate indomethacin-induced gastric ulcer in mice by improving oxidative stress, inflammation and apoptosis. J. Ethnopharmacol. **284**, 114752.

50. Salem M.B., Elzallat M., Mohammed D.M., Samir S., Hammam O.A., Abdel-Wareth M.T.A. 2023. Cornu aspersum mucin attenuates indomethacins-induced gastric ulcers in mice via alleviating oxidative stress and inflammation. Heliyon. **9**.

51. Alzokaky A.A., Abdelkader E.M., El-Dessouki A.M., Khaleel S.A., Raslan N.A. 2020. C-phycocyanin protects against ethanol-induced gastric ulcers in rats: Role of HMGB1/NLRP3/NF-κB pathway. Basic Clin. Pharmacol. Toxicol. **127**, 265–77.

52. Patel V., Joharapurkar A., Kshirsagar S., Patel M., Savsani H., Patel A., Ranvir R., Jain M. 2022. Repurposing dimethyl fumarate for gastric ulcer and ulcerative colitis: Evidence of local efficacy without systemic side effect. Med. Drug Discov. **16**, 100142.

53. Ruiz-Hurtado P.A., Garduño-Siciliano L., Dominguez-Verano P., Martinez-Galero E., Canales-Martinez M.M., Rodriguez-Monroy M.A. 2021. Evaluation of the gastroprotective effects of Chihuahua propolis on indomethacin-induced gastric ulcers in mouse. Biomed. Pharmacother. **137**, 111345.

54. Park M. et al. 2022. Zileuton Alleviates Radiation-Induced Cutaneous Ulcers via Inhibition of Senescence-Associated Secretory Phenotype in Rodents. Int. J. Mol. Sci. **23**, 8390.

55. Gilani S.J. et al. 2022. Protective effect of fustin against ethanol-activated gastric ulcer via downregulation of biochemical parameters in rats. ACS omega. **7**, 23245–54.

56. Verma S., Kumar V.L. 2018. Artesunate affords protection against aspirin–induced gastric injury by targeting oxidative stress and proinflammatory signaling. Pharmacol. Reports. **70**, 390–7.

57. Zheng H., Chen Y., Zhang J., Wang L., Jin Z., Huang H., Man S., Gao W. 2016. Evaluation of protective effects of costunolide and dehydrocostuslactone on ethanol-induced gastric ulcer in

mice based on multi-pathway regulation. Chem. Biol. Interact. **250**, 68–77.

58. Raish M., Shahid M., Bin Jardan Y.A., Ansari M.A., Alkharfy K.M., Ahad A., Abdelrahman I.A., Ahmad A., Al-Jenoobi F.I. 2021. Gastroprotective effect of sinapic acid on ethanol-induced gastric ulcers in rats: involvement of Nrf2/HO-1 and NF-κB signaling and antiapoptotic role. Front. Pharmacol. **12**, 622815.

59. Shams S.G.E., Eissa R.G. 2022. Amelioration of ethanol-induced gastric ulcer in rats by quercetin: implication of Nrf2/HO1 and HMGB1/TLR4/NF-κB pathways. Heliyon. **8**, e11159.

60. Pierri E.G., Castro R.C., Vizioli E.O., Ferreira C.M.R., Cavalheiro A.J., Tininis A.G., Chin C.M., Santos A.G. 2017. Anti-inflammatory action of ethanolic extract and clerodane diterpenes from Casearia sylvestris. Rev. Bras. Farmacogn. **27**, 495–501.

61. Liang T.-Y., Deng R.-M., Li X., Xu X., Chen G. 2021. The role of nitric oxide in peptic ulcer: a narrative review. Med. Gas Res. **11**, 42.

62. Song F., Zuo X., Zhao Y., Li Q., Tian Z., Yang Y. 2019. Betanin-enriched red beet extract attenuated platelet activation and aggregation by suppressing Akt and P38 Mitogen-activated protein kinases phosphorylation. J. Funct. Foods. **61**, 103491.

63. Chen Y., Li D., Sun L., Qi K., Shi L. 2022. Pharmacological inhibition of toll-like receptor 4 with TLR4-IN-C34 modulates the intestinal flora homeostasis and the MyD88/NF-κB axis in ulcerative colitis. Eur. J. Pharmacol. **934**, 175294.

64. Trott, O. and Olson, A.J. (2010) AutoDock Vina: Improving the Speed and Accuracy of Docking with a New Scoring Function, Efficient Optimization, and Multithreading. Journal of Computational Chemistry, 31, 455-461.

65. Gilbert, N.C., Gerstmeier, J., Schexnaydre, E.E., Borner, F., Garscha, U., Neau, D.B., Werz, O., Newcomer, M.E. (2020) Nat Chem Biol 16: 783-790).