Research Article

Gastroprotective Activity of Violacein Isolated from *Chromobacterium violaceum* on Indomethacin-Induced Gastric Lesions in Rats: Investigation of Potential Mechanisms of Action

Paulrayer Antonisamy,¹ Ponnusamy Kannan,² Adithan Aravinthan,¹
Veeramuthu Duraipandiyan,³ Mariadhas Valan Arasu,³ Savarimuthu Ignacimuthu,²
Naif Abdullah Al-Dhabi,³ and Jong-Hoon Kim¹

¹ Biosafety Research Institute, College of Veterinary Medicine, Chonbuk National University, 664-14 1GA, Duck Jin-Dong, Deokjin-gu, Jeonju City, Jeollabuk-do 561-756, Republic of Korea

² Division of Ethnopharmacology, Entomology Research Institute, Loyola College, Chennai, Tamil Nadu 600 034, India

³ Department of Botany and Microbiology, Addiriyah Chair for Environmental Studies, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia

Correspondence should be addressed to Jong-Hoon Kim; jhkim1@jbnu.ac.kr

Received 17 April 2014; Revised 18 June 2014; Accepted 26 June 2014; Published 5 August 2014

Academic Editor: Fulvio D'Acquisto

Copyright © 2014 Paulrayer Antonisamy et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Chromobacterium violaceum, Gram-negative bacteria species found in tropical regions of the world, produces a distinct deep violet-colored pigment called violacein. In the present study, we investigated whether violacein can promote a gastroprotective effect and verified the possible mechanisms involved in this action. For this study, an indomethacin-induced gastric ulcer rat model was used. The roles of biomolecules such as MPO, PGE₂, pro- and anti-inflammatory cytokines, growth factors, caspase-3, NO, K⁺ ATP channels, and α_2 -receptors were investigated. Violacein exhibited significant gastroprotective effect against indomethacin-induced lesions, while pretreatment with L-NAME and glibenclamide (but not with NEM or yohimbine) was able to reverse this action. Pretreatment with violacein also restored cNOS level to normal and led to attenuation of enhanced apoptosis and gastric microvascular permeability. Our results suggest that violacein provides a significant gastroprotective effect in an indomethacin-induced ulcer model through the maintenance of some vital protein molecules, and this effect appears to be mediated, at least in part, by endogenous prostaglandins, NOS, K⁺ ATP channel opening, and inhibition of apoptosis and gastric microvascular permeability.

1. Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) remain the first line therapy for rheumatoid arthritis and osteoarthritis. Unfortunately, their therapeutic effects are problematic due to gastrointestinal toxicity [1], primarily via inhibition of prostaglandin synthesis [2], neutrophil infiltration [3], nitric oxide imbalance [4], induction of apoptosis [5], and production of free radicals [6]. These radicals play a prominent role in microvascular injury [7] and cell death [5].

In the pharmaceutical industry, microorganisms have become an important source of natural products. Nearly

63% of commercially available drugs are directly or indirectly derived from microorganisms, plants, or animals [8]. *Chromobacterium violaceum* is a Gram-negative β -proteobacterium, facultative anaerobic, saprophyte, free-living soil- and water-associated microorganism found in water body soils in tropical and subtropical regions of the world. Violacein, a purple pigment, is a main characteristic of this bacterium and exhibits significant activity against essential tropical pathogens such as *Mycobacterium tuberculosis*, *Trypanosoma cruzi*, *Leishmania* sp., and *Plasmodium falciparum*. It is reported to have antifungal, antioxidant, antitumor, bactericidal, cytotoxic, and antiviral activities [9].

Several studies have shown that violacein is also capable of inducing apoptosis in a variety of cancer cell lines, including leukemia lineages, suggesting a promising clinical application in cancer treatment. The therapeutic application of violacein to cancer chemoprevention has been the focus of recent studies [10, 11]. Violacein has been shown to induce apoptosis in HL60 leukemic cells but is ineffective in normal human lymphocytes and monocytes [12]. In our previous studies, we showed that violacein possesses immunomodulatory, analgesic, antipyretic, antidiarrheal, and antiulcerogenic activities [13, 14].

In this study, we investigated the gastroprotective effect of violacein in an indomethacin-induced ulcer model in Wistar rats to determine its mechanisms of action.

2. Materials and Methods

2.1. Animals. Adult Wistar albino rats (200–220 g) of both sexes were used for experiments. Animals were housed with a 12 h light/dark cycle at $25 \pm 1^\circ\text{C}$ at a relative moisture of 60–70%; they had access to diet and water *ad libitum* and underwent at least two weeks of adaptation before starting the experiments. All studies were carried out using six animals in each group. All animal experiments were conducted according to the ethical norms approved by the Ministry of Social Justice and Empowerment, Government of India, and the procedures of the Institutional Animal Ethics Committee.

2.2. Chemicals and Drugs. Indomethacin, omeprazole, celecoxib, SC560, N-G-nitro-L-arginine methyl ester (L-NAME), N-ethylmaleimide (NEM), yohimbine, and glibenclamide were obtained from Sigma-Aldrich (Sigma Chemicals Co., St. Louis, MO, USA). Carboxymethyl cellulose (CMC) was obtained from Himedia (Mumbai, India). The PGE₂ EIA kit, vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), epidermal growth factor (EGF), and enzyme-linked immunosorbent assay (ELISA) kits were purchased from Cayman Chemical (Ann Arbor, MI, USA). Tumor necrosis factor (TNF)- α , interleukin (IL)-1 β , IL-4, IL-6, and IL-10 ELISA kits were purchased from Pierce Biotechnology (Rockford, IL, USA). The apoptosis assay kit was acquired from Boehringer Mannheim, and caspase-3 activity assays were conducted using the Quanti Zyme assay system from Biomol Research Laboratories, Inc. (USA). All other chemicals used were of analytical reagent grade. The test compound, violacein, was isolated from *C. violaceum* ESBV 4400, which was isolated from forest water body soil samples from Kolli Hills of Tamil Nadu, India, at latitude $11^\circ 10'$ to $11^\circ 30' \text{N}$ and longitude $78^\circ 15'$ to $78^\circ 30' \text{E}$ (Figure 1) and reported in our previous study [14].

2.3. Determination of Doses. To determine the lowest effective dose of violacein, gastric ulcers were induced by indomethacin followed by treatment with violacein. After fasting for 24 h, rats were distributed into eight groups ($n = 6/\text{group}$). Animals orally received vehicle alone (0.5 mL of 0.5% CMC) for *sham* and indomethacin treated control group, omeprazole as the positive control (40 mg/kg), or

FIGURE 1: Structure of violacein.

violacein (10, 20, 40, 80, or 160 mg/kg). After 30 min, the animals orally received 20 mg/kg of indomethacin except sham treated group. Six hours later, the animals were sacrificed under ether anesthesia, and the stomach was surgically removed, immersed in 5% formalin for 30 min, and opened along the greater curvature to macroscopically examine lesions according to the ulcer score described by previous method [15].

2.4. Gastric Damage Induced by Indomethacin. Rats ($n = 6$) were treated with *sham* (0.5 mL of 0.5% CMC), vehicle + Indo (0.5 mL of 0.5% CMC), violacein (40 mg/kg, p.o.), omeprazole (40 mg/kg p.o.), SC560 + violacein (5 mg/kg p.o. + 40 mg/kg p.o.), celecoxib + violacein (3.5 mg/kg p.o. + 40 mg/kg p.o.), L-NAME + violacein (50 mg/kg i.p. + 40 mg/kg p.o.), NEM + violacein (10 mg/kg s.c. + 40 mg/kg p.o.), yohimbine + violacein (2 mg/kg i.p. + 40 mg/kg p.o.), or glibenclamide + violacein (5 mg/kg p.o. + 40 mg/kg p.o.). All drugs were administered using 0.5% CMC as the vehicle solution. After 30 min, each group of animals except the *sham* treated group received a 20 mg/kg oral dose of indomethacin. Selective COX-1 inhibitor (SC560), COX-2 inhibitor (celecoxib), nonselective nitric oxide synthase (NOS) inhibitor (L-NAME), endogenous sulfhydryl antagonist (NEM), α_2 -receptors antagonist (yohimbine), and K⁺ATP channels antagonist (glibenclamide) were administered to rats 30 min before violacein treatment and 1 h prior to ulcer induction by indomethacin. Six hours later, animals were sacrificed under ether anesthesia, and the stomach was surgically removed, immersed in 5% formalin for 30 min, and opened along the greater curvature to macroscopically examine lesions according to the ulcer score described by previous method [15]:

- 0 = no damage;
- 1 = blood at the lumen;
- 2 = pin-point erosions;
- 3 = one to five small erosions <2 mm;
- 4 = more than five small erosions <2 mm;
- 5 = one to three large erosions >2 mm;
- 6 = more than three large erosions >2 mm.

The inhibition percentage was calculated using the following formula from Demirbilek et al.: $[(\text{UI nontreated} - \text{UI treated})/\text{UI nontreated}] \times 100$ [16].

2.5. Determination of MPO Activity Level. MPO activity in the gastric mucosa was determined according to the method described previously [17]. MPO activity in gastric tissues was expressed as $\mu\text{mol}/\text{min}/\text{mg}$ tissue.

2.6. Determination of Inflammatory Mediators and Tissue Growth Factors Levels. PGE_2 , $\text{TNF-}\alpha$, $\text{IL-1}\beta$, IL-4 , IL-6 , IL-10 , VEGF , EGF , and HGF were quantified in the stomach homogenate using enzyme-linked immunosorbent assay kits according to the manufacturer's specifications. The results were expressed as pg/gm tissue or ng/gm tissue.

2.7. Determination of NOS Activity. Gastric mucosal NOS activity was measured spectrophotometrically with the oxidation of oxyhemoglobin to methemoglobin by NO as previously described [18, 19]. The absorption difference between 401 and 421 nm was constantly observed with a dual wavelength recording spectrophotometer at 37°C . Induced NOS (iNOS) activity was calculated by subtraction of cNOS activity from total NOS activity.

2.8. Determination of Apoptosis and Caspase-3 Activity Level. Apoptosis and caspase-3 levels have been assayed spectrophotometrically by previous methods [20, 21]. Apoptosis and caspase-3 levels were expressed as unit/mg protein and as pmol/mg protein, respectively.

2.9. Determination of Microvascular Permeability. Rats were divided into six groups, each containing six animals. Animals were fasted for 24 h prior to experiments and allowed free access to water. The first group of rats received 0.5 mL of 0.5% CMC and served as *sham* treated group. The second group was subjected to gastric injury by intragastric installation of indomethacin at a dose of 20 mg/kg and was used as the ulcer-induced group. The remaining four groups were given violacein (40 mg/kg), sucralfate (400 mg/kg), SC560 + violacein (30 mg/kg + 40 mg/kg), or celecoxib + violacein (30 mg/kg + 40 mg/kg) by intragastric administration at 1 hr before ulcer induction using indomethacin. All drugs, including indomethacin, violacein, sucralfate, SC560, and celecoxib, were suspended in 0.5% CMC. Gastric microvascular permeability was evaluated 4 h after indomethacin treatment by measuring the extravasated amount of Evan's blue dye in the mucosa according to the previously mentioned method [22]. In each animal, 1 mL of 1% (w/v) Evan's blue in sterile saline was injected intravenously 30 min before sacrifice. Under ether anesthesia, animals were sacrificed by bleeding from the descending aorta, the stomachs were removed, and the gastric mucosa was scraped off and immersed in distilled water. The dye was extracted with formamide and quantified spectrophotometrically at 620 nm, and results are expressed as $\mu\text{g}/\text{mg}$ protein.

2.10. Statistical Analysis. Data were statistically analyzed using analysis of variance (ANOVA), followed by Student's *t*-test. A probability level lower than 0.05 was considered statistically significant.

3. Results and Discussion

Because of their anti-inflammatory, analgesic, and antipyretic effects, NSAIDs are commonly used to treat rheumatoid arthritis, pyrexia, bone pain, headache, migraine, acute gout, and many other conditions [23]. High dosage, inappropriate consumption, or sustained use of NSAIDs occasionally causes severe intestinal ulcer and gastroduodenal sicknesses [23]. The gastroprotective effect of violacein against NSAID-induced ulcer has not been reported until now. In the present study, we evaluated the gastroprotective effects of orally administered violacein on indomethacin-induced gastric damage in rats. Our macroscopic analyses exposed that administration of indomethacin (20 mg/kg) produced noticeable mucosal injury in the abdomen. Violacein pretreated group (Figure 2(c)) or omeprazole group (Figure 2(d)) considerably reduced gastric lesion compared to the ulcer control group, where indomethacin induced intense gastric mucosal damage in the form of elongated band of hemorrhages (Figure 2(b)). Normal group shows intact stomach without any incisions (Figure 2(a)). Regarding effective dose evaluation, the vehicle + Indo group and the 10 and 20 mg/kg groups and the 40 mg/kg dose showed a significant ulcer protective effect ($P < 0.05$). The 80 and 160 mg/kg doses of violacein produced the same effect as the 40 mg/kg dose, so 40 mg/kg was selected as the upper limit for further experiments. Rats receiving only vehicle (*sham* treated) showed no gastric mucosal lesions, while indomethacin administration produced mucosal lesions in rat stomachs. Compared with rats in untreated group, the indomethacin damage scores in violacein (40 mg/kg)—and omeprazole—treated groups were reduced by 86.39% and 88.30%, correspondingly (Figure 3).

MPO activity is known to increase in ulcerated situations and to be reduced through the curing process. MPO activity level is regularly used as a threat indicator and investigative device for evaluating the harshness of an intestinal ulcer [24]. In this study, we found that gastric MPO activity was significantly increased in the indomethacin group from $3.60 \mu\text{mol}/\text{min}/\text{mg}$ tissue (*sham* treated) to $10.72 \mu\text{mol}/\text{min}/\text{mg}$ tissue. The effect of violacein (40 mg/kg) against MPO level ($3.72 \mu\text{mol}/\text{min}/\text{mg}$ tissue) was greater than that of omeprazole ($3.91 \mu\text{mol}/\text{min}/\text{mg}$ tissue), but the difference was not statistically significant (Figure 4). NSAIDs exert their therapeutic action by inhibiting the COXs isoenzymes (COX-1 and COX-2) and reducing the levels of circulating PGs. However, the reduced levels of PGs in the intestinal mucosa are known to cause gastric ulceration and to exacerbate preexisting gastric ulcers in both rodents and humans [25]. PGs stimulate mucus and bicarbonate secretion as well as mucosal blood flow and encourage angiogenesis [24]. All of these elements contribute to reducing healing time and repairing ulcers. In this study, mucosal PGE_2 levels were markedly suppressed (3.41-fold) in indomethacin-induced

FIGURE 2: Gastroprotective activity of violacein (40 mg/kg) on indomethacin-induced gastric injury in rats. (a) Sham treated rats, (b) vehicle + indomethacin treated rats, (c) violacein (40 mg/kg) pretreated rats, and (d) omeprazole (40 mg/kg) pretreated rats. Note that indomethacin induced severe injuries to the gastric mucosa that appear as elongated bands of hemorrhage (blue arrow).

rats ($P < 0.05$) compared with sham treated group. Oral treatment with violacein and omeprazole upregulated the mucosal PGE_2 level by 3.07- and 3.24-fold, respectively (Figure 5). Pretreatment with SC560 resulted in a significant reduction in PGE_2 level in violacein-pretreated ulcerated rats. Thus, it is promising that violacein exerts its gastroprotective effect by stimulating synthesis of COX-1-derived PGE_2 . On the other hand celecoxib (COX-2 inhibitor) is unable to prevent the gastroprotective effect of violacein, which indicates that the COX-2 mediated prostaglandin synthesis was not involved in violacein activity. The present study also confirmed that the selective COX-1 inhibitor SC560 did not induce any injury on stomachs of normal rats but decreases the PGE_2 level significantly (data not shown) similar to indomethacin; this is consistent with previous observations [26, 27]. However, pretreatment of SC560 completely inhibit the gastroprotective activity of violacein against indomethacin-induced ulcer; this observation clearly elucidated that the gastroprotective activity of violacein also was facilitated through the COX-1 mediated pathways. Previous reports show that PGE_2 acts as a potential inhibitor of $\text{TNF-}\alpha$ [28, 29], apoptosis [30], and activator of K^+ ATP channels [31]; it is possible that violacein may be able to stop ulcer induction processes via the inhibition of $\text{TNF-}\alpha$, apoptosis, and activation of K^+ ATP channels through significant production of PGE_2 by COX-1 mediated pathways.

Indomethacin-induced ulcer shows increased expression of proinflammatory cytokines [32], which are associated with the degree of ulceration. Indomethacin administration elevated proinflammatory Th1 cytokines and reduced anti-inflammatory cytokines [33]. ELISA study illustrated that indomethacin stimulated $\text{TNF-}\alpha$ (2.18-fold), $\text{IL-1}\beta$ (2.11-fold), and IL-6 (1.30-fold) and downregulated IL-4 (2.59-fold), IL-10 (1.52-fold), VEGF (2.79-fold), EGF (2.26-fold), and HGF (2.49-fold) levels. Violacein at a dose of 40 mg/kg significantly ($P < 0.05$) reduced proinflammatory cytokine ($\text{TNF-}\alpha$, $\text{IL-1}\beta$, and IL-6) level (1.84-fold, 1.95-fold, and 1.45-fold, resp.) (Figure 6(a)) and increased anti-inflammatory cytokines (IL-4 and IL-10) level (2.69-fold and 2.28-fold, resp.) (Figure 6(b)) and growth factors (VEGF , EGF , and HGF) level (2.90-fold, 2.43-fold, and 2.41-fold, resp.) compared with the indomethacin-induced ulcerated untreated group (Figure 7). Overall above results explicate that the violacein treatment reduced proinflammatory cytokines ($\text{TNF-}\alpha$, $\text{IL-1}\beta$, and IL-6) and concurrently increased the levels of tissue IL-4 and IL-10 , all of which may contribute to its healing effect.

$\text{TNF-}\alpha$ seems to be a crucial contributor to many forms of intestinal mucosal injury, including that during the application of NSAIDs. NSAIDs have been shown to markedly elevate the level of $\text{TNF-}\alpha$. Inhibition of $\text{TNF-}\alpha$ production results in attenuation of the harmful effects of NSAIDs in the rat intestine [34]. Prostaglandins are potent inhibitors

FIGURE 3: Effect of violacein (10, 20, 40, 80, and 160 mg/kg, orally) on indomethacin-induced ulcer index in rats. Values are mean $\pm$ SD ($n = 6$). * $P < 0.05$ compare vehicle + Indo with all the groups. Values in the braces indicate ulcer index inhibition percentage. Indo: indomethacin; Vio: violacein; UI: ulcer index; ns: nonsignificant.

FIGURE 4: Effect of COX-1 and COX-2 inhibitors and violacein (40 mg/kg, orally) on MPO level in indomethacin-induced ulcer. Values are mean $\pm$ SD ($n = 6$). * $P < 0.05$ compare vehicle + Indo with all the groups; # $P < 0.05$ compare Vio (40 mg) + Indo with SC560 (or) Celecoxib + Vio (40 mg) + Indo. Indo: indomethacin; Vio: violacein; MPO: myeloperoxidase.

of $\text{TNF-}\alpha$ release from both macrophages [29] and mast cells [28]. IL-10 has a central role in the downregulation of the inflammatory cascade by depressing the production of a number of proinflammatory cytokines [35] and enhancing the production of anti-inflammatory cytokines [36]. In this study violacein enhances IL-10 and inhibited $\text{TNF-}\alpha$ significantly, which clearly indicates the ulcer curing ability of violacein on NSAID induced ulcer. VEGF is a growth factor that increases ulcer healing by stimulating angiogenesis

FIGURE 5: Effect of violacein (40 mg/kg, orally) on mucosal PGE_2 level in indomethacin-induced ulcer. Values are mean $\pm$ SD ($n = 6$). * $P < 0.05$ compare vehicle + Indo with all the groups; # $P < 0.05$ compare Vio (40 mg) + Indo with SC560 (or) Celecoxib + Vio (40 mg) + Indo. Indo: indomethacin; Vio: violacein.

[37]. Likewise, HGF aids angiogenesis, through multiple mechanisms including COX activation, and increases EGF expression that eventually accelerates gastroduodenal ulcer healing by increasing gastric mucin and diminishing gastric acid secretion [38]. We determined that indomethacin administration significantly decreased mucosal VEGF, EGF, and HGF levels compared with the vehicle-treated control group, but violacein treatment considerably enriched growth factors levels. After COX-1 inhibitor pretreatment, ulcer index (UI) and MPO level were significantly increased followed by reduction of tissue VEGF, EGF, HGF, IL-4, IL-10, and PGE_2 levels. However, the COX-2 inhibitor celecoxib did not affect the therapeutic activity of violacein at any level ($P < 0.05$).

Gastric mucosal cNOS activity was significantly decreased in the indomethacin-induced ulcer group. In contrast, the iNOS activity in the gastric mucosa of rats subjected to indomethacin induction was significantly increased [39]. Treatment with violacein significantly increased cNOS and decreased iNOS, but this activity was inhibited by L-NAME, a nonspecific inhibitor, which confirms the involvement of NOS in violacein-mediated gastroprotection on indomethacin-induced ulcers (Figure 8).

Nonprotein endogenous sulfhydryl (NP-SH) compounds are important for the maintenance of gastric mucosal integrity [40]. These SH groups have the ability to bind to the free radicals generated by noxious agents, thus controlling the production and nature of mucus [40]. However, our results showed significant attenuations in the gastric ulcer area after the blockage of NP-SH compounds by NEM in groups treated with violacein (40 mg/kg) in comparison with the indomethacin-induced ulcer group, suggesting that the gastroprotective effects of violacein are not involved in maintenance of NP-SH compounds (Figure 9). The opening of K^+ ATP channels, a class of ligand-gated proteins, appears to be involved with a variety of physiologic functions of

FIGURE 6: (a) Effect of violacein (40 mg/kg, orally) on proinflammatory cytokines (TNF- α , IL-1 β , and IL-6) level; (b) anti-inflammatory cytokines (IL-4 and IL-10) level in indomethacin-induced ulcer. Values are mean $\pm$ SD ($n = 6$). * $P < 0.05$ compare vehicle + Indo with all the groups; # $P < 0.05$ compare Vio (40 mg) + Indo with SC560 (or) Celecoxib + Vio (40 mg) + Indo. Indo: indomethacin; Vio: violacein.

FIGURE 7: Effect of COX-1 and COX-2 inhibitors and violacein (40 mg/kg, orally) on VEGF, EGF, and HGF levels in indomethacin-induced ulcerated rats. Values are mean $\pm$ SD ($n = 6$). * $P < 0.05$ compare vehicle + Indo with all the groups; # $P < 0.05$ compare Vio (40 mg) + Indo with SC560 or Celecoxib + Vio (40 mg) + Indo. Indo: indomethacin; Vio: violacein.

FIGURE 8: Effect of L-NAME (NOS inhibitor) and violacein (40 mg/kg, orally) on cNOS, tNOS, and iNOS levels in indomethacin-induced ulcerated rats. Values are mean $\pm$ SD ($n = 6$). * $P < 0.05$ compare vehicle + Indo with all the groups; # $P < 0.05$ compare Vio (40 mg) + Indo with L-NAME + Vio (40 mg) + Indo. Indo: indomethacin; Vio: violacein; L-NAME: N-G- nitro- L-arginine methyl ester.

the stomach, such as gastric blood flow regulation, acid secretion, and stomach contractility [41]. In fact, Peskar et al. demonstrated that endogenous prostaglandins act as activators of K^+ ATP channels, and this mechanism, at least in part, mediates gastroprotection [42]. A previous study by Peskar et al. suggested the participation of K^+ ATP channels in an indomethacin-induced ulcer model in which

prostaglandins were shown as probable activators of these channels [42]. In this way, our results showed that the gastroprotection mechanism of violacein was K^+ ATP-channel dependent, since its gastroprotective effects were reverted by pretreatment with glibenclamide, a potent antagonist of these

FIGURE 9: Effect of L-NAME (NOS inhibitor), NEM (NP-SH antagonist), yohimbine (α_2 -receptors antagonist), glibenclamide (K^+ ATP-channels antagonist), SC560 (COX-1 inhibitor), and Celecoxib (COX-2 inhibitor) on gastroprotective activity of violacein against indomethacin-induced ulcer. Values are mean $\pm$ SD ($n = 6$). * $P < 0.05$ compare vehicle + Indo with all the groups; # $P < 0.05$ compare Vio (40 mg) + Indo with L-NAME, NEM, YO, GLIB, SC560, or Celecoxib + Vio (40 mg) + Indo. Values in the braces indicate ulcer index inhibition percentage. Indo: indomethacin; Vio: violacein; L-NAME: N-G-nitro-L-arginine methyl ester; NEM: N-ethylmaleimide; YO: yohimbine; GLIB: glibenclamide.

FIGURE 10: (a) Effect of violacein (40 mg/kg, orally) on apoptosis level; (b) caspase-3 level in indomethacin-induced ulcerated rats. Values are mean $\pm$ SD ($n = 6$). * $P < 0.05$ compare Vehicle + Indo with all the groups. Values in the braces indicate apoptosis and caspase-3 level inhibition percentage. Indo: indomethacin; Vio: violacein.

channels (Figure 9). From these data, we suggest participation of K^+ ATP channels in the gastroprotective effects of violacein, in which prostaglandins could be involved in the activation of these channels. Presynaptic α_2 -receptors mediate several responses in the gastrointestinal tract and are involved in the regulation of gastric acid secretion [43]. Pretreatment with the α_2 -receptor antagonist yohimbine failed to effectively block the gastroprotective effect of violacein (40 mg/kg) against indomethacin-induced ulcers (Figure 9), suggesting that the gastroprotective effect of violacein is not mediated by α_2 -receptors. L-NAME significantly inhibited the gastroprotection produced by violacein (Figure 9), suggesting that NO participates in gastroprotection. It is well known that NO is

involved in the modulation of gastric mucosal integrity and in the regulation of acid and alkaline secretion, mucus secretion, and gastric mucosal blood flow [44].

Enhancement of caspase-3 activation and considerable epithelial cell apoptosis are important pathological events during NSAIDs-induced cytotoxicity [33]. The amplification and propagation of the cell death signaling cascade induced by TNF- α involve the activation of a family of specific cysteine proteases known as caspases, which are under the regulatory control of nitric oxide [45]. Indomethacin displays its effects on apoptogenic signal propagation through the induction of TNF- α [46]; our present experiments showed that violacein significantly inhibited TNF- α . The apoptotic

FIGURE 11: Effect of COX-1 and COX-2 inhibitors and violacein (40 mg/kg, orally) on gastric microvascular permeability induced by indomethacin in rats. Values are mean $\pm$ SD ($n = 6$). * $P < 0.05$ compare vehicle + Indo with all the groups; # $P < 0.05$ compare Vio (40 mg) + Indo with SC560 or Celecoxib + Vio (40 mg) + Indo. Indo: indomethacin; Vio: violacein.

index in the indomethacin-induced group was 10.48-fold higher than that in the normal control group. Pretreatment with violacein caused a 65.27% reduction in DNA fragmentation (Figure 10(a)). The caspase-3 activity in the indomethacin-induced group was 3.55-fold higher than in the sham treated group. Pretreatment with violacein caused a 52.26% reduction in caspase-3 activity (Figure 10(b)). Based on these results, it is possible that violacein may be able to inhibit these apoptogenic processes by the inhibition of TNF- α . The effects of violacein, sucralfate, SC560, and celecoxib on the indomethacin-induced microvascular permeability of rat stomach are depicted in Figure 11. Indomethacin increased gastric microvascular permeability by 4.2-fold. Both violacein and sucralfate ameliorated indomethacin-induced gastric microvascular permeability (73.68% and 74.12%, resp.). Microvascular permeability was significantly increased after COX-1 inhibitor pretreatment along with violacein; however, the COX-2 inhibitor celecoxib did not affect the therapeutic activity of violacein (40 mg/kg). The above result indicates the involvement of COX-1 in the process of inhibition of vascular permeability. These results are consistent with a previous report [27].

The results of our present study demonstrate that violacein presented significant gastroprotective effects in an indomethacin-induced gastric ulcer model and that appear to be mediated, at least in part, by endogenous prostaglandins, nitric oxide, K^+ ATP channel opening, upregulation of the levels of mucosal growth factors, maintenance of the balance of pro- and anti-inflammatory cytokines, antiapoptotic function, and attenuation of enhanced gastric microvascular permeability. These findings indicate that violacein may be a useful natural gastroprotective tool. However, further studies are required to evaluate the exact mechanism involved in

its action as well as to better investigate the safety profile of violacein use.

Conflict of Interests

The authors declare no competing financial interests.

Acknowledgment

The authors thank Addiriyah Chair for Environmental Studies, Department of Botany and Microbiology, College of Science, King Saud University, P.O. Box 2455, Riyadh, Saudi Arabia, for the support.

References

- [1] M. R. Griffin and J. M. Scheiman, "Prospects for changing the burden of nonsteroidal anti-inflammatory drug toxicity," *The American Journal of Medicine*, vol. 110, no. 1, pp. 33S–37S, 2001.
- [2] U. S. Akarca, "Gastrointestinal effects of selective and non-selective non-steroidal anti-inflammatory drugs," *Current Pharmaceutical Design*, vol. 11, no. 14, pp. 1779–1793, 2005.
- [3] J. L. Wallace, C. M. Keenan, and D. N. Granger, "Gastric ulceration induced by nonsteroidal anti-inflammatory drugs is a neutrophil-dependent process," *The American Journal of Physiology—Gastrointestinal and Liver Physiology*, vol. 259, no. 3, pp. G462–G467, 1990.
- [4] B. L. Slomiany, J. Piotrowski, and A. Slomiany, "Role of endothelin-1 and constitutive nitric oxide synthase in gastric mucosal resistance to indomethacin injury: effect of antiulcer agents," *Scandinavian Journal of Gastroenterology*, vol. 34, no. 5, pp. 459–464, 1999.
- [5] H. Kusuha, H. Komatsu, H. Sumichika, and K. Sugahara, "Reactive oxygen species are involved in the apoptosis induced by nonsteroidal anti-inflammatory drugs in cultured gastric cells," *European Journal of Pharmacology*, vol. 383, no. 3, pp. 331–337, 1999.
- [6] H. Utsumi, K. Yasukawa, T. Soeda et al., "Noninvasive mapping of reactive oxygen species by in vivo electron spin resonance spectroscopy in indomethacin-induced gastric ulcers in rats," *The Journal of Pharmacology and Experimental Therapeutics*, vol. 317, no. 1, pp. 228–235, 2006.
- [7] Y. Naito and T. Yoshikawa, "Oxidative stress involvement and gene expression in indomethacin-induced gastropathy," *Redox Report*, vol. 11, no. 6, pp. 243–253, 2006.
- [8] D. J. Newman and G. M. Cragg, "Microbial antitumor drugs: natural products of microbial origin as anticancer agents," *Current Opinion in Investigational Drugs*, vol. 10, no. 12, pp. 1280–1296, 2009.
- [9] N. Durán and C. F. M. Menck, "*Chromobacterium violaceum*: a review of pharmacological and industrial perspectives," *Critical Reviews in Microbiology*, vol. 27, no. 3, pp. 201–222, 2001.
- [10] P. S. Melo, G. Z. Justo, M. B. M. de Azevedo, N. Durán, and M. Haun, "Violacein and its β -cyclodextrin complexes induce apoptosis and differentiation in HL60 cells," *Toxicology*, vol. 186, no. 3, pp. 217–225, 2003.
- [11] V. S. Saraiva, J. Marshall, J. Cools-Lartigue, and M. N. Burnier Jr., "Cytotoxic effects of violacein in human uveal melanoma cell lines," *Melanoma Research*, vol. 14, no. 5, pp. 421–424, 2004.

- [12] C. V. Ferreira, C. L. Bos, H. H. Versteeg, G. Z. Justo, N. Durán, and M. P. Peppelenbosch, "Molecular mechanism of violacein-mediated human leukemia cell death," *Blood*, vol. 104, no. 5, pp. 1459–1464, 2004.
- [13] P. Antonisamy and S. Ignacimuthu, "Immunomodulatory, analgesic and antipyretic effects of violacein isolated from *Chromobacterium violaceum*," *Phytomedicine*, vol. 17, no. 3–4, pp. 300–304, 2010.
- [14] P. Antonisamy, P. Kannan, and S. Ignacimuthu, "Anti-diarrhoeal and ulcer-protective effects of violacein isolated from *Chromobacterium violaceum* in Wistar rats," *Fundamental and Clinical Pharmacology*, vol. 23, no. 4, pp. 483–490, 2009.
- [15] J. B. Dekanski, A. Macdonald, P. Sacra, and D. V. Parke, "Effects of fasting, stress and drugs on gastric glycoprotein synthesis in the rat," *British Journal of Pharmacology*, vol. 55, no. 3, pp. 387–392, 1975.
- [16] S. Demirbilek, I. Gürses, N. Sezgin, A. Karaman, and N. Gürbüz, "Protective effect of polyunsaturated phosphatidylcholine pretreatment on stress ulcer formation in rats," *Journal of Pediatric Surgery*, vol. 39, no. 1, pp. 57–62, 2004.
- [17] P. P. Bradley, D. A. Priebe, R. D. Christensen, and G. Rothstein, "Measurement of cutaneous inflammation: estimation of neutrophil content with an enzyme marker," *Journal of Investigative Dermatology*, vol. 78, no. 3, pp. 206–209, 1982.
- [18] M. Feelisch and E. A. Noack, "Correlation between nitric oxide formation during degradation of organic nitrates and activation of guanylate cyclase," *European Journal of Pharmacology*, vol. 139, no. 1, pp. 19–30, 1987.
- [19] R. G. Knowles, M. Merrett, M. Salter, and S. Moncada, "Differential induction of brain, lung and liver nitric oxide synthase by endotoxin in the rat," *Biochemical Journal*, vol. 270, no. 3, pp. 833–836, 1990.
- [20] B. L. Slomiany and A. Slomiany, "Platelet-activating factor mediates *Helicobacter pylori* lipopolysaccharide interference with gastric mucin synthesis," *IUBMB Life*, vol. 56, no. 1, pp. 41–46, 2004.
- [21] B. L. Slomiany and A. Slomiany, "Nitric oxide as a modulator of gastric mucin synthesis: role of ERK and p38 mitogen-activated protein kinase activation," *IUBMB Life*, vol. 54, no. 5, pp. 267–273, 2002.
- [22] C. L. Chander, A. R. Moore, F. M. Desa, D. Howat, and D. A. Willoughby, "The local modulation of vascular permeability by endothelial cell derived products," *Journal of Pharmacy and Pharmacology*, vol. 40, no. 10, pp. 745–746, 1988.
- [23] R. Simone, *Australian Medicines Handbook*, Australian Medicines Handbook, Adelaide, Australia, 2006.
- [24] M. K. Jones, H. Wang, B. M. Peskar et al., "Inhibition of angiogenesis by nonsteroidal anti-inflammatory drugs: insight into mechanisms and implications for cancer growth and ulcer healing," *Nature Medicine*, vol. 5, no. 12, pp. 1418–1423, 1999.
- [25] F. Halter, A. S. Tarnawski, A. Schmassmann, and B. M. Peskar, "Cyclooxygenase 2: implications on maintenance of gastric mucosal integrity and ulcer healing: controversial issues and perspectives," *Gut*, vol. 49, no. 3, pp. 443–453, 2001.
- [26] B. Gretzer, N. Maricic, M. Respondek, R. Schuligoi, and B. M. Peskar, "Effects of specific inhibition of cyclo-oxygenase-1 and cyclo-oxygenase-2 in the rat stomach with normal mucosa and after acid challenge," *British Journal of Pharmacology*, vol. 132, no. 7, pp. 1565–1573, 2001.
- [27] A. Tanaka, S. Hase, T. Miyazawa, R. Ohno, and K. Takeuchi, "Role of cyclooxygenase (COX)-1 and COX-2 inhibition in nonsteroidal anti-inflammatory drug-induced intestinal damage in rats: relation to various pathogenic events," *The Journal of Pharmacology and Experimental Therapeutics*, vol. 303, no. 3, pp. 1248–1254, 2002.
- [28] C. M. Hogaboam, E. Y. Bissonnette, B. C. Chin, A. D. Befus, and J. L. Wallace, "Prostaglandins inhibit inflammatory mediator release from rat mast cells," *Gastroenterology*, vol. 104, no. 1, pp. 122–129, 1993.
- [29] S. L. Kunkel, M. Spengler, M. A. May, R. Spengler, J. Larrick, and D. Remick, "Prostaglandin E₂ regulates macrophage-derived tumor necrosis factor gene expression," *Journal of Biological Chemistry*, vol. 263, no. 11, pp. 5380–5384, 1988.
- [30] H. Sheng, J. Shao, J. D. Morrow, R. D. Beauchamp, and R. N. DuBois, "Modulation of apoptosis and Bcl-2 expression by prostaglandin E₂ in human colon cancer cells," *Cancer Research*, vol. 58, no. 2, pp. 362–366, 1998.
- [31] A. S. Gomes, L. M. F. Lima, C. L. Santos, F. Q. Cunha, R. A. Ribeiro, and M. H. L. P. Souza, "LPS from *Escherichia coli* protects against indomethacin-induced gastropathy in rats—role of ATP-sensitive potassium channels," *European Journal of Pharmacology*, vol. 547, no. 1–3, pp. 136–142, 2006.
- [32] T. Brzozowski, P. C. Konturek, S. J. Konturek et al., "Classic NSAID and selective cyclooxygenase (COX)-1 and COX-2 inhibitors in healing of chronic gastric ulcers," *Microscopy Research and Technique*, vol. 53, no. 5, pp. 343–353, 2001.
- [33] B. L. Slomiany, J. Piotrowski, and A. Slomiany, "Role of caspase-3 and nitric oxide synthase-2 in gastric mucosal injury induced by indomethacin: effect of sucralfate," *Journal of Physiology and Pharmacology*, vol. 50, no. 1, pp. 3–16, 1999.
- [34] C. B. Appleyard, D. McCafferty, A. W. Tigley, M. G. Swain, and J. L. Wallace, "Tumor necrosis factor mediation of NSAID-induced gastric damage: Role of leukocyte adherence," *The American Journal of Physiology—Gastrointestinal and Liver Physiology*, vol. 270, no. 1, pp. G42–G48, 1996.
- [35] P. Stordeur and M. Goldman, "Interleukin-10 as a regulatory cytokine induced by cellular stress: molecular aspects," *International Reviews of Immunology*, vol. 16, no. 5–6, pp. 501–522, 1998.
- [36] M. A. Cassatella, L. Meda, S. Gasperini, F. Calzetti, and S. Bonora, "Interleukin 10 (IL-10) upregulates IL-1 receptor antagonist production from lipopolysaccharide-stimulated human polymorphonuclear leukocytes by delaying mRNA degradation," *Journal of Experimental Medicine*, vol. 179, no. 5, pp. 1695–1699, 1994.
- [37] A. Tarnawski, I. L. Szabo, S. S. Husain, and B. Soreghan, "Regeneration of gastric mucosa during ulcer healing is triggered by growth factors and signal transduction pathways," *Journal of Physiology—Paris*, vol. 95, no. 1–6, pp. 337–344, 2001.
- [38] T. Brzozowski, P. C. Konturek, S. J. Konturek et al., "Involvement of cyclooxygenase (COX)-2 products in acceleration of ulcer healing by gastrin and hepatocyte growth factor," *Journal of Physiology and Pharmacology*, vol. 51, no. 4, pp. 751–773, 2000.
- [39] H. Matsuda, Y. Li, and M. Yoshikawa, "Roles of capsaicin-sensitive sensory nerves, endogenous nitric oxide, sulfhydryls, and prostaglandins in gastroprotection by momordin Ic, an oleanolic acid oligoglycoside, on ethanol-induced gastric mucosal lesions in rats," *Life Sciences*, vol. 65, no. 2, pp. 27–32, 1999.
- [40] M. A. Andreo, K. V. R. Ballesteros, C. A. Hiruma-Lima, L. R. Machado da Rocha, A. R. M. Souza Brito, and W. Vilegas, "Effect of *Mouriri pusa* extracts on experimentally induced gastric lesions in rodents: role of endogenous sulfhydryls

- compounds and nitric oxide in gastroprotection,” *Journal of Ethnopharmacology*, vol. 107, no. 3, pp. 431–441, 2006.
- [41] M. L. Garcia, M. Hanner, H. Knaus et al., “Pharmacology of potassium channels,” *Advances in Pharmacology*, vol. 39, pp. 425–471, 1997.
- [42] B. M. Peskar, K. Ehrlich, and B. A. Peskar, “Role of ATP-sensitive potassium channels in prostaglandin-mediated gastroprotection in the rat,” *The Journal of Pharmacology and Experimental Therapeutics*, vol. 301, no. 3, pp. 969–974, 2002.
- [43] K. Gyires, K. Müllner, S. Fürst, and A. Z. Rónai, “Alpha-2 adrenergic and opioid receptor-mediated gastroprotection,” *Journal of Physiology Paris*, vol. 94, no. 2, pp. 117–121, 2000.
- [44] P. Kubes, M. Suzuki, and D. N. Granger, “Nitric oxide: an endogenous modulator of leukocyte adhesion,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 88, no. 11, pp. 4651–4655, 1991.
- [45] A. Parenti, L. Morbidelli, X. Cui et al., “Nitric oxide is an upstream signal of vascular endothelial growth factor-induced extracellular signal-regulated kinase 1/2 activation in post capillary endothelium,” *The Journal of Biological Chemistry*, vol. 273, no. 7, pp. 4220–4226, 1998.
- [46] D. Wallach, “Cell death induction by TNF: a matter of self control,” *Trends in Biochemical Sciences*, vol. 22, no. 4, pp. 107–109, 1997.