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Prickly Pear Cactus (*Opuntia ficus indica* var. *saboten*) Protects Against Stress-Induced Acute Gastric Lesions in Rats

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ABSTRACT The protective activity of prickly pear cactus (*Opuntia ficus indica* var. *saboten*) fruit juice and its main constituent, betanin, were evaluated against stress-induced acute gastric lesions in rats. After 6 h of water immersion restraint stress (WIRS), gastric mucosal lesions with bleeding were induced in Sprague–Dawley rats. Pretreatment of a lyophilized powder containing *O. ficus indica* var. *saboten* fruit juice and maltodextrin (OFSM) and betanin significantly reduced stress lesions (800–1600 mg/kg). Both OFSM and betanin effectively prevented the decrease in gastric mucus content as detected by alcian blue staining. In addition, OFSM significantly suppressed WIRS-induced increases in the level of gastric mucosal tumor necrosis factor-α and myeloperoxidase (MPO). Betanin alone was only effective in decreasing MPO. These results revealed the protective activity of OFSM against stress-induced acute gastric lesions and that betanin may contribute to OFSM's gastric protective activity, at least in part. When OFSM and betanin were taken together, OFSM exerted gastroprotective activity against stress-induced gastric lesions by maintaining gastric mucus, which might be related to the attenuation of MPO-mediated damage and proinflammatory cytokine production.

KEY WORDS: • ***betanin*** • ***gastric lesion*** • **Opuntia ficus indica *var.* saboten** • ***water immersion restraint stress***

INTRODUCTION

OPUNTIA FICUS INDICA (Cactaceae) grows in all the semiarid countries throughout the world and is especially cultivated in the Mediterranean region and in Central America. Both its fruits and cladodes have been used in traditional medicine of many countries. In addition, there have been extensive studies on the biological activities of this plant.[1,2] Particularly, antiulcerogenic activities of its fruits and cladodes against ethanol-induced injury have been reported.[3,4]

O. ficus indica has been presumed to be introduced as an ornament plant to Korea. Since then it has been naturalized as *O. ficus indica* var. *saboten* and is widely cultivated in Jeju Island, Korea, for use in the manufacture of health foods, such as tea, jam, and juice.[5,6] The dried powder of its fruits and stems significantly inhibited HCl-ethanol–, HCl-aspirin–, and indomethacin-induced gastric lesions in rats without any change in gastric juice secretion.[7,8] Its bioactive chemical constituents include several flavonoids that have been isolated from this plant.[6] Moreover, betanin is a principal purple-red color pigment in the fruits of *Opuntia* species. Betanin has recently been reported to possess antioxidant activity.[1]

It is well known that both physical and psychological stresses can cause gastric ulceration in humans and experimental animals. Water immersion restraint stress (WIRS) is widely accepted for studying stress ulcers and can also mimic the clinical acute gastric ulcerations caused by trauma, surgery, or sepsis.[9] It has been reported that oxidative stress generated by infiltrated neutrophils and the xanthine-xanthine oxidase system and its subsequent lipid peroxidation is involved in the development of gastric mucosal lesions induced by WIRS.[10] WIRS is also known to provoke acute inflammation in gastric mucosa. This inflammation is accompanied by increased expression of several proinflammatory cytokines, including tumor necrosis factor-α (TNF-α), that seem to be responsible for enhanced permeability of blood vessels to neutrophils.[11] Since both *O. ficus indica* fruit and betanin showed antioxidative and anti-inflammatory activities,[12,13] they have great potential to be effective against WIRS-induced gastric damage. Thus, in the present study, the effect of O. *ficus indica* var. *saboten* fruit juice was evaluated on WIRS-induced gastric lesions. In addition, betanin was tested in combination with *O. ficus*

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indica var. *saboten* to determine whether it could contribute to the gastric protective activity of *O. ficus indica* var. *saboten* fruit juice.

MATERIALS AND METHODS

Plant material

O. ficus indica var. *saboten* fruits collected during the autumn season in Jeju Island, Korea, were purchased from a local market. The fruits were peeled and finely chopped. The pulp was separated from the seeds and crushed in a grinding mill. The obtained mash was filtered and then sterilized at 85°C for 20 min. The extracted juice was mixed with maltodextrin (dextrose equivalent 16.5–19.5; Sigma-Aldrich) at a ratio of 7.5:1 and freeze-dried.

Quantitation of betanin in fruit juice

The lyophilized powder containing *O. ficus indica* var. *saboten* fruit juice and maltodextrin (OFSM) was solubilized in 50% methanol at the concentration of 200 mg/mL. Stock solution of betanin (TCI America) was prepared in 50% methanol at the concentration of 2 mg/mL and the appropriate amount of every standard solution was mixed and diluted with 50% methanol as indicated. Analytical methods for the determination of betanin were optimized by testing various conditions in the high-performance liquid chromatography (HPLC) system consisting of a chromatographic pump (P680, Dionex), an injector (7725i; Rheodyne), and a photodiode array (DAD; UVD 340U, Dionex). The output signal of the detector was recorded using a Dionex Chromelon™ Chromatography Data System. Chromatographic separation was achieved on Shiseido CAPCELL PAK C18 MG (5 μm, 4.6 mm i.d. $\times$ 150 mm) by gradient elution of a mixture of 0.5% acetic acid containing acetonitrile (A) and water (B) at a flow rate of 1.0 mL/min (0 min, 100% of B; 10 min, 88% of B; 20 min, 80% B), and monitored at 535 nm.

Animals

Male Sprague–Dawley rats (7 weeks old) (Orient Co. Ltd.) were adapted for a week to the controlled environment (20–23°C; 12 h light cycle from 09:00 to 21:00; food, Agribrand Purina Korea, and water *ad libitum*). After the adaptation, rats weighing 250–300 g were used for the experiments. All the experiments were conducted according to the guidelines of the Committee on Care and Use of Laboratory Animals of Seoul National University.

WIRS-induced gastric lesions

OFSM was solubilized in 0.5% carboxy methyl cellulose (CMC) to the desired concentration just before use. The rats were randomly divided into six groups: normal (no treatment, no WIRS exposure), control (5 mL of 0.5% CMC/kg), Stillen™ (350 mg/kg), OFSM I (800 mg/kg), OFSM II (1600 mg/kg), and betanin (280 mg/kg) groups ($n = 8$ rats per group, treatment administered orally). Stillen, a prescription drug for gastric ulcer approved by Korean Food and Drug Administration, was used as a positive control. Stillen is also a standardized extract of medicinal herbs, *Artemisia asiatica*, and has been reported to have potent protective activities against various gastric lesions in animal models.[14] WIRS was induced according to the methods of Kang *et al.*[15] and An *et al.*[16] with minor modifications. Briefly, all the samples were administered to the rats after withholding food for 18 h. The animals received 0.5% CMC or other test materials 30 min before they were placed in individual restraint cages and vertically immersed in a water bath (20°C) to the level of the xyphoid process for 6 h.

Determination of gastric lesion index

After 6 h exposure to the stress, the animals were sacrificed under ether anesthesia, and the stomach was removed and opened along the greater curvature. The total area of gastric mucosal erosions was measured and the degree of gastric mucosal injury was expressed as the gastric lesion index (mm^2).[17]

Gastric adherent mucus assay

The level of mucus bound to the gastric epithelial surface was determined according to the method of Kang *et al*.[15] Briefly, the glandular portion of the stomach was excised and immersed for 2 h in 0.1% Alcian blue in a sucrose solution. The unbound dye was then removed by two subsequent washings, and the mucus-bound dye was eluted by immersing the stomach in 30% docusate sodium salt solution for 2 h. After centrifugation of the eluent, the optimal density of the Alcian blue solution was read at 620 nm, and calculated using the calibration curve. The results were expressed as the concentration of Alcian blue adhering to the gastric mucosal surface (μg/g tissue).

Measurement of mucosal myeloperoxidase and TNF-α

The scraped mucosa was homogenized and the membrane-bound myeloperoxidase (MPO) was solubilized. The homogenate was sonicated, thawed, and centrifuged. The supernatant was used to determine gastric mucosal MPO concentration using an ELISA kit (HK105-02 rat MPO kit; Hycult Biotech) according to the manufacturer's instruction. The concentration of TNF-α in the tissue homogenized supernatant was measured using an ELISA kit specific for rat TNF-α (Quantikine® immunoassay kit; R&D Systems).

Measurement of the level of lipid peroxidation

The level of lipid peroxidation was determined by thiobarbituric acid reactive substances assay according to Animal Models of Diabetic Complications Consortium protocols (see www.amdcc.org). Briefly, the scraped mucosa was homogenized in lysis buffer (Pro-Pre; iNtRON Biotechnology) and reacted with 10% trichloroacetic acid to precipitate protein. Then, samples were incubated for 15 min on ice and centrifuged for 15 min at 2200 *g*, 4°C. An

equal volume of 0.67% (w/v) 2,4,6-tribromoanisole was added to the supernatant and the mixed sample was incubated in a boiling water bath for 10 min. After cooling, the absorbance was measured at 532 nm with an Infinite M200 Spectrophotometer (Tecan Ltd.).

Statistical analysis

All data were presented as mean ± standard error of mean (SEM). Statistical analyses were performed with one-way analysis of variance, followed by Dunnett's *post hoc* test. P values < .05 were considered statistically significant.

RESULTS

Determination of betanin content

A new analytical method employing HPLC-DAD showed appropriate specificity for betanin in this study. The linearity of betanin was determined from six concentrations ranging from 0.5 to 40.0 mg/mL. The regression equation was calculated as $y = 0.0797x - 0.7284$ with a high correlation coefficient value ($r^2 = 0.9995$), where y and x were the values of peak area and the amount of betanin, respectively. Three batches of freeze-dried OFSM were used to determine the content of betanin and their average content was 17.6 ± 0.8% (w/w).

Effect of OFSM and betanin on WIRS-induced gastric lesions

Six hours of WIRS loading produced significant hemorrhagic injury in the glandular stomach without any significant difference in body weight between groups. OFSM at oral doses of 800 (OFSM I) and 1600 mg/kg (OFSM II) dose-dependently prevented stress-induced gastric injury (Fig. 1). Quantitatively, OFSM pretreatment significantly reduced gastric lesion index from 239.5 to 106.3 mm^2 and 87.8 mm^2 at doses of 800 and 1600 mg/kg, respectively (Fig. 2). According to the above mentioned analytical results, 1600 mg of OFSM can be calculated to contain about 280 mg of betanin. Thus, to evaluate the contribution of betanin to the gastric protective activity of OFSM, 280 mg/kg body weight of betanin was orally administered to the rats. Betanin (130.5 mm^2), and although it was less effective than OFSM at the dose used in this study, also effectively prevented WIRS-induced gastric lesion and its efficacy was comparable to that of Stillen (140.4 mm^2), a positive control (Fig. 2).

Effect of OFSM and betanin on the levels of adherent mucus

Gastric mucosa is continuously exposed to many noxious factors and substances. A continuous layer of mucus covers gastric mucosa and serves as a physiological barrier and primary preepithelial defense.[15] Rats with WIRS had a significant decrease in gastric adherent mucus concentration when compared with unstressed normal rats. Preadministration of OFSM significantly attenuated the decreased gastric adherent mucus concentration and it was more efficient at the higher dose (1600 mg/kg) (Fig. 3). Interestingly, samples from the rats treated with betanin restored gastric adherent mucus concentration almost to the level of 1600 mg/kg of OFSM, implying that betanin might be one of the major contributors to the effect of OFSM on gastric adherent mucus.

Effect of OFSM and betanin on the levels of MPO, TNF-α, and lipid peroxidation

An assay of gastric mucosal MPO was used to quantify the degree of neutrophil infiltration. To evaluate anti-inflammatory and antioxidative activities of OFSM and betanin in our experimental conditions, the level of TNF-α and lipid peroxidation was measured together. The WIRS-induced increase in MPO concentration in the gastric mucosa was significantly suppressed by OFSM and betanin (Fig. 4). There was no significant difference between the effectiveness of OFSM and betanin on MPO. The gastric mucosal

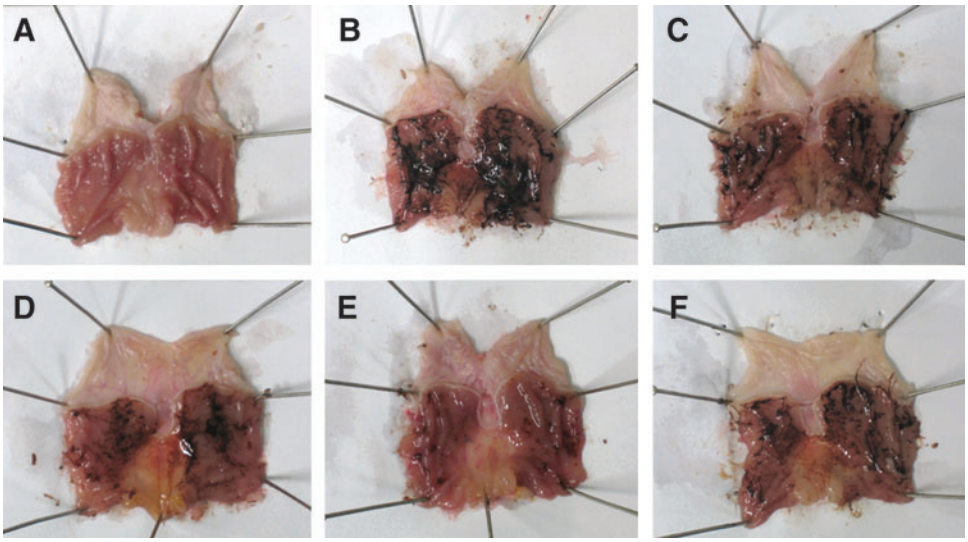

FIG. 1. Appearance of gastric mucosal lesions induced by WIRS. The gastric mucosa of a normal rat **(A)**, and rats with 6 h of WIRS alone **(B)**, and rats with 6 h of WIRS and pretreatment of Stillen (350 mg/kg) **(C)**, OFSM (I; 800 mg/kg) **(D)**, OFSM (II; 1600 mg/kg) **(E)**, and betanin (280 mg/kg) **(F)**. WIRS, water immersion restraint stress; OFSM, lyophilized powder containing *Opuntia ficus indica* var. *saboten* fruit juice and maltodextrin. Color images available online at www.liebertpub.com/jmf

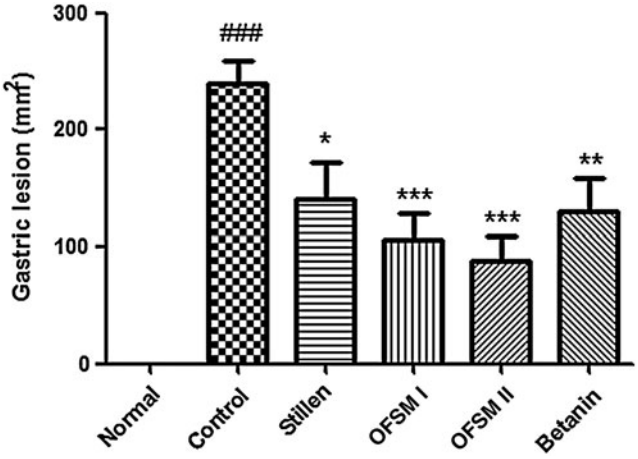

FIG. 2. Effect of pretreatment of OFSM and betanin on gastric mucosal lesions induced by WIRS. Groups OFSM I and OFSM II were orally administered 800 mg/kg and 1600 mg/kg of OFSM, respectively. Each value is a mean ± SEM of eight animals in each group. $^{###}P < .001$, compared with normal rats without WIRS; $^{*}P < .05$, $^{**}P < .01$, and $^{***}P < .001$, compared with rats with WIRS alone. SEM, standard error of the mean.

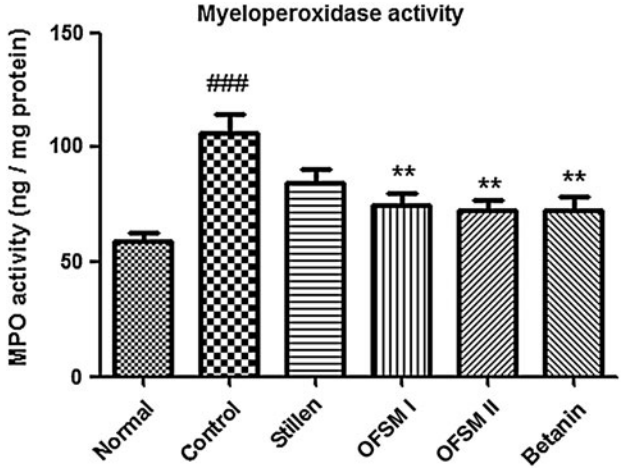

FIG. 4. Effect of pretreatment of OFSM and betanin on gastric mucosal concentration of MPO. Groups OFSM I and OFSM II were orally administered 800 mg/kg and 1600 mg/kg of OFSM, respectively. Each value is a mean ± SEM of eight animals in each group. $^{###}P < .001$, compared with normal rats without WIRS; $^{**}P < .01$, compared with rats with WIRS alone. MPO, myeloperoxidase.

concentration of TNF-α was also increased significantly after 6 h of WIRS. OFSM at 1600 mg/kg showed the most potent suppressive effect on WIRS-induced increase in TNF-α. With regard to betanin treatment, there was no statistically significant decrease in TNF-α, although the mean value of TNF-α concentration was lower than that of stress-loaded control rats (Fig. 5). Since both *Opuntia* species and betanin are well known for their antioxidative activities,[1,6] the level of lipid peroxidation was estimated in the rats pretreated with OFSM or betanin 1 h before WIRS loading. Despite the expectations, only the positive control, Stillen (0.016 nmol/mg protein malondialdehyde), significantly reduced lipid peroxidation induced by WIRS. The malondialdehyde concentrations in the samples from the rats treated with 800 mg/kg OFSM, 1600 mg/kg OFSM, and betanin were 0.033, 0.030, and 0.038 nmol/mg protein, respectively (Fig. 6). When compared with the level of WIRS-loaded control group, 0.043 nmol/mg protein, those values were relatively lower. However, there was no statistically significant difference between the values.

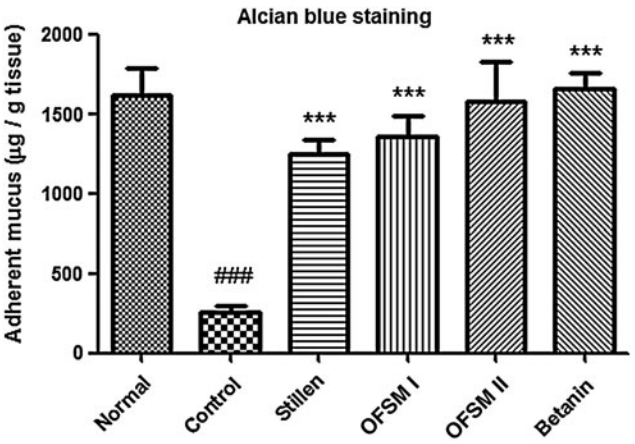

FIG. 3. Effect of pretreatment of OFSM and betanin on adherent mucus concentration in gastric mucosa. Groups OFSM I and OFSM II were orally administered 800 mg/kg and 1600 mg/kg of OFSM, respectively. Each value is a mean ± SEM of eight animals in each group. $^{###}P < .001$, compared with normal rats without WIRS; $^{***}P < .001$, compared with rats with WIRS alone.

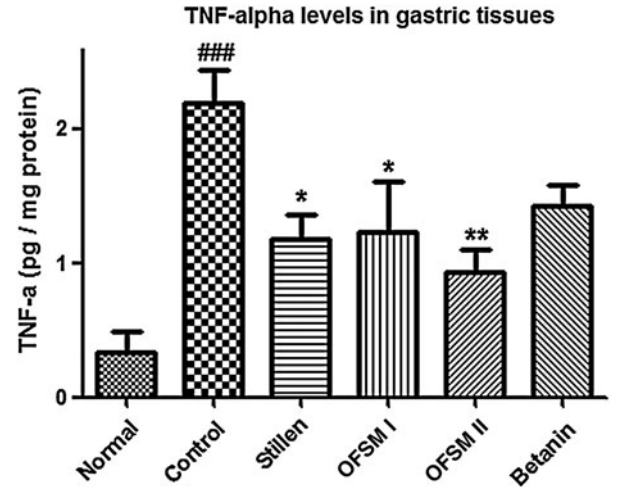

FIG. 5. Effect of pretreatment of OFSM and betanin on gastric mucosal concentration of TNF-α. Groups OFSM I and OFSM II were orally administered 800 mg/kg and 1600 mg/kg of OFSM, respectively. Each value is a mean ± SEM of eight animals in each group. $^{###}P < .001$, compared with normal rats without WIRS; $^{*}P < .05$ and $^{**}P < .01$, compared with rats with WIRS alone.

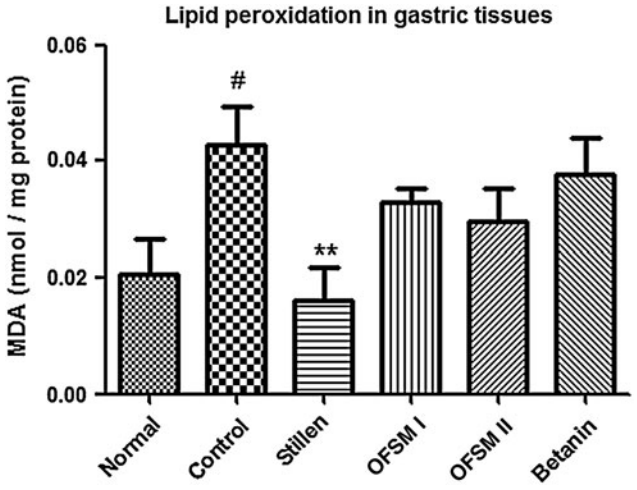

FIG. 6. Effect of pretreatment of OFSM and betanin on the level of gastric mucosal lipid peroxidation. Groups OFSM I and OFSM II were orally administered 800 mg/kg and 1600 mg/kg of OFSM, respectively. Each value is a mean ± SEM of eight animals in each group. $^{\#}P < .05$, compared with normal rats without WIRS; $^{**}P < .01$, compared with rats with WIRS alone.

DISCUSSION

Stress has long been recognized as an important risk factor for a variety of illness, ranging from mental illness to gastrointestinal disorders, although there is considerable ambiguity about the definition of stress. A wide variety of experimental animal models have been developed to mimic human stressful events and to unravel its underlying mechanisms. Among all experimental-stress-inducing methods, those characterized by rodents exposed to an unavoidable, physical stress are the most extensively used.[18] In the present study, WIRS was employed to test the protective effect of the fruits of *O. ficus indica* var. *saboten* against stress-induced gastric lesions, since it is a widely used experimental model to induce acute stress ulcers in rats and is known for its reliable reproducibility.[19]

A large number of natural products have been evaluated for their antiulcler effects while searching for possible candidates for new drugs or functional foods.[20] *Opuntia* species are also well known for its gastroprotective activities as demonstrated in various experimental models.[7,8] Since the fruits of *O. ficus indica* var. *saboten* have not been tested in stress-induced gastritis model, we studied its effect on WIRS in rats.

To improve the homogeneity and hygroscopicity, the fruit juice of *O. ficus indica* var. *saboten* was mixed with maltodextrin, dried, and lyophilized. In a preliminary test, this powder sample (OFSM) did not show significant gastroprotective activity below 800 mg/kg in the WIRS model. At dosages above 1600 mg/kg, there was no dose-dependent increase in protective activity. Thus, we chose two doses, 800 mg/kg (OFSM I) and 1600 mg/kg (OFSM II), to study further their gastroprotective effects. Moreover, betanin, one of the major pigments in OFSM, was also tested at 280 mg/kg to determine whether it is the main bioactive constituent in OFSM. The dose was calculated from OFSM II, which is the minimum dose for the highest activity. None of these samples exhibited any sign of toxicity, including weight loss.

In WIRS experiments, disturbance of gastric mucosal microcirculation is implicated in gastric lesions, which is followed by impaired gastric mucus synthesis and secretion.[21] In our experimental conditions, significant decrease of adherent mucus was observed after 6 h of WIRS. However, it was restored by pretreatment of OFSM and betanin (Fig. 3). Since gastric mucus plays a critical role in the primary defense of the gastric mucosa and provides a protective barrier in the gastric epithelium,[19] these results suggest gastroprotective activity of OFSM and betanin, at least in part, through preservation of gastric mucus synthesis and secretion.

MPO is an enzyme found primarily in the azurophilic granules of neutrophils and is used as a marker for quantification of neutrophil sequestration in tissue and the severity of inflammation.[22] This enzyme also has enormous potential to inflict tissue damage through its ability to catalyze the production of a complex array of reactive oxidants.[13] Previously, betanin exhibited significant inhibitory effects on human MPO activity.[13] Accordingly, OFSM and betanin effectively inhibited WIRS-stimulated MPO activity in our experimental conditions (Fig. 4), which implies the possible contribution of betanin to the action of OFSM through its antioxidative and anti-inflammatory properties.

Increase in plasma TNF-α level after several hours of WIRS in rats has been reported in several studies.[15,23,24] TNF-α, a major proinflammatory cytokine, plays an important role in the development of acute inflammation, mediated by neutrophil infiltration of gastric mucosa.[23] In Kwiencién's study,[23] pretreatment with pentoxyfilline, an inhibitor of TNF-α activity, resulted in a significant reduction in gastric lesions. The decreased gastric lesion by nitric oxide–releasing aspirin, when compared with aspirin, was accompanied by decreases in plasma TNF-α.[24] Although, it is not certain whether TNF-α is a cause or a result of gastroprotection, these studies suggest the involvement of TNF-α-mediated inflammatory cascade in WIRS-induced gastric lesion. Based on these observations, the effect of OFSM on plasma TNF-α was evaluated. As a result, OFSM reduced plasma TNF-α, while betanin did not show significant activity on TNF-α (Fig. 5). These results suggest the possibilities of the presence of other active constituents in OFSM.

Taken together, OFSM and its major constituent, betanin, significantly prevented WIRS-induced gastric lesions in rat mainly by preservation of gastric mucus.

CONCLUSIONS

Oral administration of OFSM could effectively prevent serious gastric lesions induced by WIRS. It restored the level of gastric mucosal adherent mucus decreased by WIRS. The WIRS-induced increases in MPO and TNF-α were significantly suppressed by OFSM. Although betanin, one of the major constituents of OFSM, was relatively less potent when compared with OFSM, it also showed similar effects.

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AUTHOR DISCLOSURE STATEMENT

The authors declare that there are no conflicts of interest.

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