Hesperidin protects against stress induced gastric ulcer through regulation of peroxisome proliferator activator receptor gamma in diabetic rats

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A B S T R A C T

Stress induced gastric ulcer is a serious health problem in diabetic patients. Some studies reported that hesperidin (HDN), a citrus bioflavonoid, can bind to and stimulate peroxisome proliferator-activator receptor-gamma (PPAR-γ) which may mediate its antidiabetic, anti-inflammatory and anti-oxidant effects. This work aims to study the possible protective effect of HDN against stress induced gastric ulcer in diabetic rats as well as the possible involvement of PPARγ in this effect. Type 2 diabetes was induced using streptozotocin and nicotinamide. Diabetic rats received either HDN (100 mg/kg/day, orally) & omeprazole (20 mg/kg/day, orally) or HDN (100 mg/kg/day, orally) + GW9662, PPARγ antagonist, (1 mg/kg/day, i.p.) for 8 weeks then acute gastric injury was induced by cold restraint stress technique. Glycemic controls and gastroprotective effects were evaluated by measuring serum levels of glucose and insulin, gastric free and total acidity and gastric ulcer indices. Histopathological examination of gastric mucosa was also performed. To determine the underlying mechanism of action, gastric mucosal expression of nuclear factor (erythroid-derived 2)-like 2 (Nrf2), hemeoxygenase-1 (HO-1), cluster of differentiation 45 (CD45), cyclooxygenase-2 (COX-2), nuclear factor kappa B (NFκB) and inducible nitric oxide synthase (iNOS), gastric contents of reduced glutathione (GSH), malondialdehyde (MDA), tumor necrosis factor alpha (TNF-α) and nitric oxide (NO); as well as superoxide dismutase (SOD) and catalase activities were measured. HDN significantly improved glycemic level; it also reduced gastric acidity and gastric ulcer index and histopathological changes comparable to that produced by omeprazole. Moreover, HDN reduced lipid peroxidation and inflammatory markers levels and enhanced antioxidant capacity. The use of GW9662 significantly abrogated the gastric protective effect of HDN as well as reduced the antioxidant and anti-inflammatory effects. Our work showed, for the first time that, HDN has promising protective effect against stress induced gastric ulcer in diabetic rats through activation of PPARγ.

1. Introduction

Gastric ulceration is an erosion in the gastric mucosa that can be induced by *Helicobacter pylori*, alcohol consumption, nonsteroidal anti-inflammatory drugs (NSAIDs), or exposure to stressful conditions [1].

Diabetes mellitus increases the susceptibility of gastric mucosa to ulcerogens and impairs gastric ulcer healing in patients with long-standing diabetes mellitus [2] through development of autonomic neuropathy [3].

Stress -induced gastric ulcer, the focus of the current study, is a serious health problem in diabetic patients which increases the length of stay in intensive care units (ICUs). Most guidelines recommend prophylactic therapy such as proton pump inhibitors (PPIs) or histamine receptor type 2 blockers (H₂R blockers). However, both treatments are associated with potential adverse effects [4]. Therefore,

investigating new treatments with lower side effects is recommended.

Stress induced gastric ulcer can be experimentally induced by restraining animals at 4 °C for 3.5 h, a technique known as cold restraint stress [5] which stimulates release of catecholamines, activation of hypothalamus-pituitary-adrenal (HPA) axis and release of cortisol [6]. Activation of HPA is associated with disorganization of gastric hormones, generation of free radicals and upregulation of oxidative stress in gastric mucosa [7]. Moreover, cortisol, when elevated slightly, enhances production of tumor necrosis factor-α (TNF-α) and interlukin-6 (IL-6) by macrophages [8]. These stressors are more aggravated in diabetic patients who suffer from a state of low grade inflammation [9] leading to more profound erosions in gastric mucosa when subjected to ulcerogenic stimuli [2]. Therefore, treatment of gastric ulcers in diabetic patients is more problematic than in non-diabetics.

Hesperidin (HDN) is a citrus bioflavonoid with promising anti-

inflammatory, anti-oxidant, anti-cancer, lipid lowering, neuroprotective and hypoglycemic effects. Several studies have reported that the anti-inflammatory effects of HDN are mediated by several mechanisms including deactivation of inducible isoforms of cyclooxygenases and nitric oxide synthases, suppression of inflammatory cytokines and inhibition of hypoxia inducible factor-1 alpha [10]. Moreover, HDN enhances the proliferation of T-lymphocytes and inhibits B cell activation [11,12]. On the other hand, the anti-oxidant effects of HDN are attributed to free radical scavenging activity, neutralization of reactive oxygen species (ROS), and augmentation of cellular anti-oxidant defenses [13].

Previous studies have reported that HDN possesses antidiabetic activity. The hypoglycemic effect of HDN is mediated by stimulating hepatic glycolysis, elevating glycogen concentration, and reduction of hepatic gluconeogenesis [14].

Peroxisome proliferator activator receptor gamma (PPARγ) is a transcription factor which can modulate inflammation, cell proliferation and differentiation [15]. Previous reports showed a potential role for PPARγ in protection against gastric ulcer [16]. Also, HDN was reported to protect against ischemic heart diseases and cyclophosamide induced hepatotoxicity via PPARγ–dependent pathways [17-19].

Relying on the aforementioned, this work aimed to study the possible protective effect of HDN against stress-induced gastric ulcer in diabetic rats compared to omeprazole (OMP, standard treatment). Furthermore, we investigated the possible involvement of PPARγ in HDN activities.

2. Materials and methods

2.1. Animals and ethics statement

Adult male Wistar rats weighting 160–190 g were purchased from the Faculty of Veterinary Medicine, Zagazig University, Egypt. Experiments on rats were started after one week acclimatization period. Rats were distributed three/cage. Diet and water were available all the time. Temperature, humidity and light/dark cycles were kept constant at the following values ($23 \pm 2\,°C$, $60 \pm 10\%$ and 12/12 h respectively). All animal handling procedures were approved by the Ethical Committee for Animal Handling at the Faculty of Pharmacy, Zagazig University, Egypt, with approval no. P5-8-2017.

2.2. Drugs and experimental design

HDN, OMP, streptozotocin (STZ), nicotinamide (NA) and GW9662 were obtained from Sigma (St. Louis, MO).

The rats were randomly divided into seven equal groups (n = 8). The first group was the non-diabetic rats, without induction of gastric ulceration, and served as control. The second group was the non-diabetic rats received saline for 8 weeks then gastric ulcer was induced (cold restrain group). The animals of the third group were rendered diabetics and received saline for 8 weeks following successful induction of diabetes, and then subjected to gastric ulcer induction (diabetic + cold restrain group). The animals of the fourth group were rendered diabetics and received OMP (20 mg/kg/day, orally, dissolved in saline) [20] for 8 weeks then subjected to stress gastric ulcer induction (diabetic + cold restrain + OMP). The animals of the fifth group were rendered diabetics and received GW9662 (1 mg/kg/day, intraperitoneal (i.p.), dissolved in saline) [17,18] for 8 weeks following successful induction of diabetes, and then subjected to gastric ulcer induction (diabetic + cold restrain + GW9662). The animals of the sixth group were rendered diabetics and received HDN (100 mg/kg/day, orally, dissolved in saline) [21] for 8 weeks [22] then subjected to gastric ulcer induction (diabetic + cold restrain + HDN). The animals of the seventh group were rendered diabetics and received GW9662 (1 mg/kg/day, i.p.) 15 min prior to HDN administration (100 mg/kg/day, orally) for 8 weeks then stress gastric ulcer was induced

(diabetic + cold restrain + HDN + GW9662).

2.3. Diabetes induction

Rats were fasted overnight and received a single dose of STZ (60 mg/kg, i.p., dissolved in 0.1 M citrate buffer, pH 4.5), 15 min following a single dose of NA (110 mg/kg, i.p., dissolved in normal saline) to induce type 2 diabetes (T2D) [23].

The control and cold restrain groups were injected with the vehicle of streptozotocin (0.1 M citrate buffer, pH 4.5).

Rats, with blood glucose levels more than 250 mg/dl on the seventh day after STZ injection, were considered diabetic. Blood glucose levels were measured using blood glucose meter (Accu-Chek, Roche Diagnostics, Mannheim, Germany). Body weights of all rats were measured at the beginning and at the end of the experiment.

2.4. Gastric ulcer induction

Gastric ulcer was induced in the respective animals after 8 weeks of the treatment. Drug administration was initiated just after the successful induction of diabetes (on the seventh day). Cold restraint stress was used to induce gastric ulcer by keeping rats immobile at low temperature (4 ± 1) for 3.5 h, before final blood and tissues collection, in individual restraint boxes without possibility of visual contact [24]. This regimen has been reported to induce gastric ulcers reliability in food-deprived rats [5]. To avoid diurnal variations, all experiments were performed at the same time of the day.

2.5. Collection of blood and serum separation

After 3.5 h of ulcer induction, all animals were anesthetized with urethane (1.3 g/kg, i.p.). Blood samples were obtained from the orbital sinus of fasted rats. Fasting blood glucose levels were measured by the blood glucose meter. Remaining blood samples were centrifuged to separate serum and stored at $-20\,°C$ until use.

2.6. Assessment of gastric mucosal lesions and tissue sampling

Stomachs were isolated and opened along the greater curvature then washed with ice-cold saline. Macroscopic mucosal lesions were examined by an observer unaware of the experiment design. Ulcer index was used to express gastric mucosal lesions [25]. Gastric mucosal lesions were scored as follows: 1 for small petechiae; and 2 to 5 for lesions of 2–5 mm length. The mean ulcer index for each group was calculated using the following equation: (sum of total scores/number of animals).

2.7. Measurement of gastric acid secretion

Gastric acidity was performed as earlier described by Ref. [26]. Twenty-four hours after the induction of gastric ulcer, the rats were sacrificed under anesthesia with urethane (1.3 g/kg, i.p.); the abdomen was opened to remove the stomach. The stomach was opened along the greater curvature and gastric content was drained into a centrifuge tube. 5 ml of distilled water was added and the resultant solution was centrifuged at 3000 rpm for 10 min. The pH of gastric juice was determined using a pH meter (Microfield, pH S-25 pH meter, England).

The free and total acid content of the gastric juice was determined by titrating gastric juice with 0.01 N NaOH, using topfer's reagent and phenolphthalein as indicator and was expressed as Meq/L/24 h [27,28]. A burette was setup in the laboratory, 0.01 N NaOH was prepared and poured into the burette. 50 ml of distilled water was added to the gastric content (aliquot) inside the plain bottles. 25 mL of gastric juice was pipetted into a beaker and three drops of Topfer's reagent added to make up for the free acid. The NaOH inside the burette was titrated against the acidic solution in the beaker, and observed until