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Some Biochemical Changes Followed Experimental Gastric Ulceration

A. H. El-Far, R. R. Gindi, H. A. Abd El-Maksoud, Mohamed Ragaa Ragab Hassanien

***Abstract*—**Gastric ulceration is a discontinuity in gastric mucosa, usually occurs due to imbalance between the gastric mucosal protective factors, that is called gastric mucosal barrier, and the aggressive factors, to which the mucosa is exposed. This study was carried out on sixty male Sprague-Dowely rats (12- 16 weeks old) allocated into two groups. The first control group and the second Gastric lesion group which induced by oral administration of a single daily dose of aspirin at a dose of 300 mg/kg body weight for 7 consecutive-days (6% aspirin solution will be prepared and each rat will be given 5 ml of that solution/kg body weight). Blood is collected 1, 2 and 3 weeks after induction of gastric ulceration. Significant increase in serum copper, nitric oxide, and prostaglandin E_2 all over the period of experiment. Significant decrease in erythrocyte superoxide dismutase (t-SOD) activities, serum (calcium, phosphorus, glucose and insulin) levels. Non-significant changes in serum sodium and potassium levels are obtained.

***Keywords*—**Aspirin, Gastric Ulcer, Prostaglandin E_2, Superoxide dismutase

I. Introduction

GASTRIC ulcer is multi-etiologic and widespread disease. It is defined as a discontinuity in the gastric mucosa penetrating through the muscularis mucosa. Small or shallow breaches which are confined to the mucosa are termed erosions. Both peptic ulcer and erosion differ from gastritis which is inflammation of the gastric mucosa [1].

Peptic ulcer is a sore that forms in the lining of the stomach or the duodenum. A peptic ulcer results from an imbalance between some endogenous aggressive factors (such as hydrochloric acid, pepsin, refluxed bile, leukotrienes, reactive oxygen species (ROS)) and cytoprotective factors, which include the function of the mucus-bicarbonate barrier, surface active phospholipids, prostaglandins (PGs), mucosal blood flow, cell renewal and migration, non-enzymatic and enzymatic antioxidants and some growth factors [2].

The pathogenesis of ulcers is multifactorial and includes diverse factors such as a stressful lifestyle, alcohol consumption, use of steroidal and non-steroidal anti-inflammatory drugs (NSAIDs) and drugs which stimulate gastric acid and pepsin secretion, Helicobacter pylori infections, smoking, lower socioeconomic status and family history [3].

El-Far, A. H. was with Department of Biochemistry, Faculty of Veterinary Medicine, Damanhour Univ., Egypt. (ali.elfar@damanhour.edu.eg).

Gindi, R.R. was with Clinicopathological Department, Faculty of Medicine, Benha University, Egypt.

Abd El-Maksoud H.A. and Mohamed Ragaa Ragab Hassanien were with Department of Biochemistry, Faculty of Veterinary Medicine, Moshtohor, Benha Univ., Egypt

The most important causes of gastric ulceration are non-steroidal anti-inflammatory drugs (NSAIDs), which cause a 'chemical gastritis', which is characterized by mucosal hyperplasia and edema but little inflammatory cells, were infiltrated [4]. Although the traditional theories regarding the pathogenesis of peptic ulcers focus on acid hypersecretion, and non-sterodial anti-inflammatory drugs (NSAIDs) which disrupt the normal mucosal defense and repair, making the mucosa more susceptible to the attack of acid [5].

Nitric oxide (NO) is influencing the biochemical and physiological reactions that are key to preventing or repairing injury to the gastrointestinal tract, as stimulating mucus secretion from the mucus membrane of stomach and intestine and regulating the blood flow in the wall of the gastrointestinal tract and the mucus membrane and control inflammatory cell activation in the inflammatory process [6].

Aspirin is one of the most commonly used NSAID associating with gastrointestinal effects of variable severities ranging from mild dyspepsia to severe fatal gastric bleeding. Leads to inhibition in the gastric mucosal protective factors and at the same time it the aggressive factors to which the mucosa of the stomach is exposed [7]. The pathogenesis of NSAIDs-induced gastric ulceration includes the block of cyclooxygenase (COX) activity that leads to lower mucus and bicarbonate secretion, decreased mucosal blood flow, neutrophil infiltration, alteration of microvascular structures, and increase of acid and pepsinogen secretion. In addition, increased production of reactive oxygen species (ROS), increased lipid peroxidation, and neutrophil infiltration have demonstrated to play a role in the pathogenesis of NSAIDs-induced ulcers, including the aspirin-induced ulcer [8].

The present study was conducted to study the biochemical effect of experimentally induced acute gastric ulceration in rats on serum nitric oxide, prostaglandin E_2, copper, calcium, phosphorus, glucose, insulin, sodium and potassium and erythrocyte, total superoxide dismutase (t-SOD).

II. Material and Methods

A. Experimental Animals

Sixty Male Sprague-Dowely rats, 12-16 weeks old weighting about 250-300 grams were used in the experimental investigation of this study. The animals were fed on a normal laboratory diet carbohydrate (58%), protein (17.5%), lipid (3.4%), cellulose (3.1%), minerals (1.49%), calcium (0.9%), phosphorus (0.59%), moisture (12%) and fibers (3.02%). All groups were given drinking water *ad lib.*

After one week of acclimatization, the animals were allotted into two groups; Group 1: Control group (30 rats in three replicates ten in each) These animals will receive 5 ml/kg body weight of 2% gum acacia orally daily for 3 days as for coating the gastric mucosa and preventing the effect of gastric

HCl; and Group 2: Gastric lesion group (30 rats in three replicates ten in each) In this group, animals will received gum acacia as control group then the gastric ulcers will be induced by oral administration of a single daily dose of aspirin® received as a powder from El-Naser company for Pharmacentrical Chemical Drugs Easily soluble in water at a dose of 300 mg/kg body weight for 7 consecutive-days (6% aspirin solution will be prepared and each rat will be given 5 ml of that solution/kg body weight) according to [9].

B. Sample collections

Blood is collected 1, 2 and 3 weeks after induction of gastric ulceration then divided into two portions the first one poured in tubes contain 20 IU heparin as anti-coagulant/1 ml blood used for preparation of hemolysated by digitonin as described by [10] after washing erythrocytes by physiological saline, this hemolysated used for estimation of erythrocytes total superoxide dismutase (t-SOD).

The second portion collected in centrifuge tubes and incubated for coagulation then centrifuged; the serum was carefully separated from the clot and used freshly for determination of serum nitric oxide [11].

Prostaglandin E_2 and insulin by using immunoradiometric assay (IRMA) according to [12], Serum calcium and phosphorus [13], glucose [14], sodium and potassium [15] and copper by atomic absorption spectrophotometry according to [16]. In addition, statistical analysis of the recorded data was analyzed according to [17].

III. Results

The recorded data in Table I showed very high significant increase (P<0.001) in the mean value of serum copper through the experimental period. While serum calcium and phosphorus decreased significantly (P<0.05) at the 2nd and 3rd week of experiment. Whereas serum sodium and potassium showed non-significant changes on comparison with the mean values of control one.

The recorded data in Table II high (p< 0.01) and very high (p< 0.001) significant increase in the mean values of nitric oxide and prostaglandin E_2, respectively in induced gastric ulceration groups while erythrocytes t-SOD decreased significantly (P<0.05) and serum glucose and insulin showed non-significant changes all over the period of experiment.

TABLE I
Mean Value±S.E of Serum Copper, Calcium, Phosphorus, Sodium And Potassium, in Control And Experimentally Induced Gastric Ulceration Groups

Group	Control	Gastric ulceration group		
Parameter		1st week	2nd week	3rd week
Copper (µlU/ml)	118.31 ±11.61	369.71 ±14.90 ***	348.18 ±15.11 ***	299.00 ±16.01 ***
Calcium (mg/dl)	12.87 ±0.59	8.28 ±0.67	7.30 ±0.81 *	6.40 ±0.91 *
Phosphorus (mg/dl)	6.87 ±0.88	4.76 ±0.97	3.98 ±0.82*	3.80 ±0.98 *
Sodium (mEq./L)	130.80 ±3.80	138.11 ±2.89	148.16 ±3.38	151.16 ±3.90
Potassium (mEq./L)	4.11 ±0.70	5.16 ±0.81	5.85 ±0.79	6.29 ±0.81

S.E: Standard Error
*: Significant at p< 0.05
**: High Significant at p< 0.01
***: Very high Significant at p< 0.001

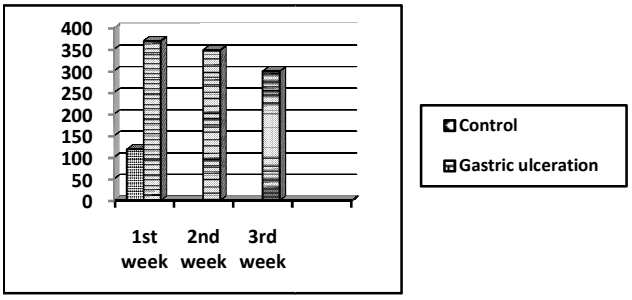

Fig. 1 Mean value±S.E of serum copper (µlU/ml) in control and experimentally induced gastric ulceration groups

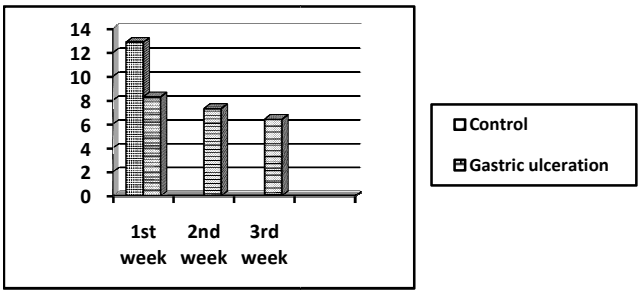

Fig. 2 Mean value±S.E of serum calcium (mg/dl) in control and experimentally induced gastric ulceration groups

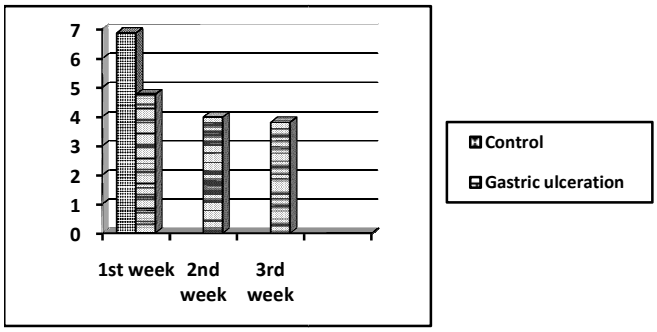

Fig. 3 Mean value±S.E of serum phosphorus (mg/dl) in control and experimentally induced gastric ulceration groups

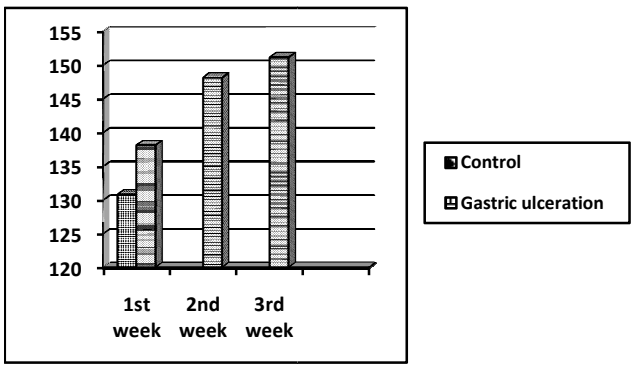

Fig. 4 Mean value±S.E of serum sodium (mEq./L) in control and experimentally induced gastric ulceration groups

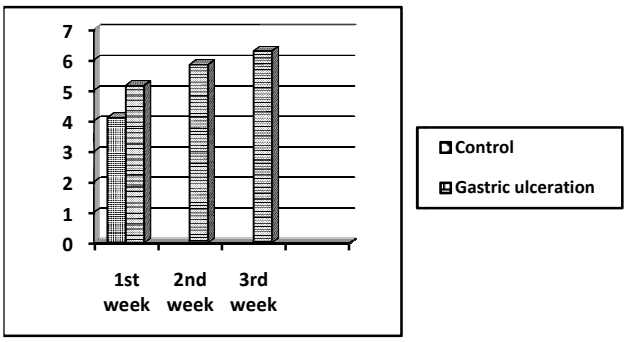

Fig. 5 Mean value±S.E of serum potassium (mEq./L) in control and experimentally induced gastric ulceration groups

TABLE II
MEAN VALUE±S.E OF SERUM NITRIC OXIDE LEVEL, PROSTAGLANDIN (E_2), ERYTHROCYTIC SUPEROXIDE DISMUTASE, GLUCOSE AND INSULIN LEVEL IN CONTROL AND EXPERIMENTALLY INDUCED GASTRIC ULCERATION GROUPS

Group / Parameter	Control	Gastric ulceration group 1st week	2nd week	3rd week
Nitric Oxide (µmol/ml)	3.90 ± 1.89	9.80 ± 3.16 **	28.75 ±3.38 **	4.86 ± 3.88 **
PGE_2 (ng/ml)	8.81 ± 2.91	49.16 ± 16.98***	918.86 ±18.88** *	72.80 ± 13.00***
Erythrocytic superoxide dismutase (µlU/ml)	.66 ± 0.48	.18 ± 0.48 *	1.37 ±0.60 *	.82 ± 0.46 *
Glucose (mg/dl)	8.18 ± 3.19	8.51 ± 4.19	80.16 ±3.38	8.16 ± 5.10
Insulin (µlU/ml)	2.19 ± 0.98	3.26 ± 0.88	12.81 ±1.06	2.08 ± 1.26

S.E: Standard Error
*: Significant at p< 0.05
**: High Significant at p< 0.01
***: Very high Significant at p< 0.001

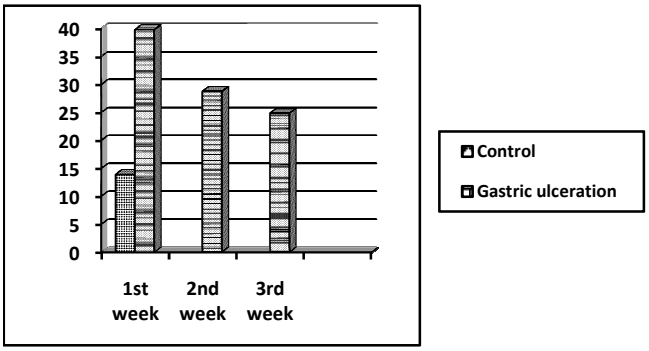

Fig. 6 Mean value±S.E of serum nitric oxide (µmol/ml) in control and experimentally induced gastric ulceration groups

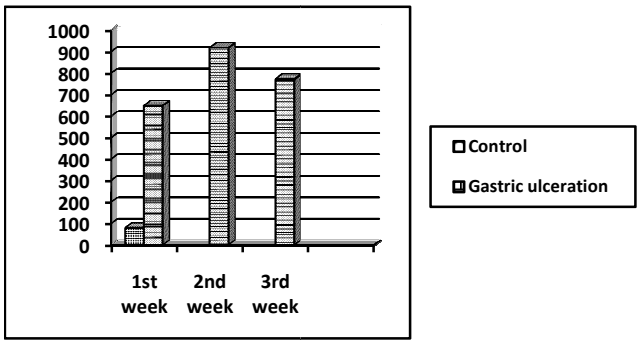

Fig. 7 Mean value±S.E of serum prostaglandin E_2 (ng/ml) in control and experimentally induced gastric ulceration groups

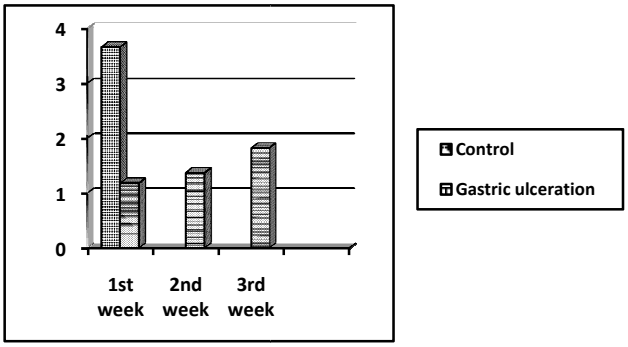

Fig. 8 Mean value±S.E of erythrocytic superoxide dismutase (µlU/ml) in control and experimentally induced gastric ulceration groups

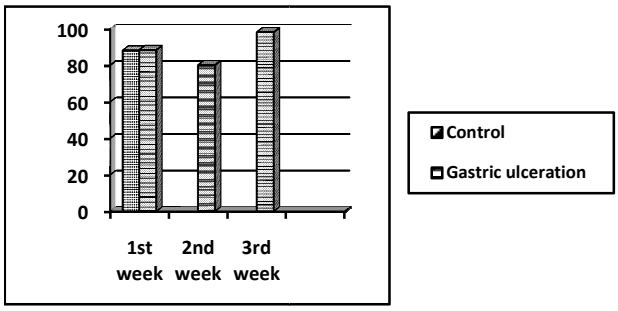

Fig. 9 Mean value±S.E of serum glucose (mg/dl) in control and experimentally induced gastric ulceration groups

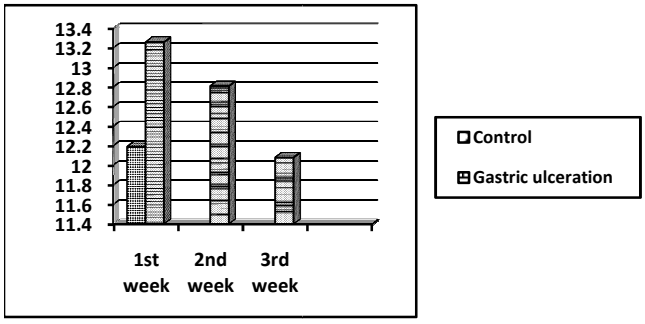

Fig. 10 Mean value±S.E of serum insulin (µlU/ml) in control and experimentally induced gastric ulceration groups

IV. Discussion

Ulcer disease whatever gastric or duodenal are the most prevalent still unresolved gastrointestinal disorders in clinical practice that faces many patients worldwide from both sexes in a wide range of age, including infancy and childhood, but are most common among middle-aged adults [18].

The present data in Table (1) revealed that the experimentally induction of gastric ulceration followed up by highly significant increase of serum copper level on comparison with the mean value in the control group that due to copper ions are essential for: the viability and maintenance of connective tissue metabolism, vascular and immunological functions, protection against oxygen radical-induced tissue destruction, and regulations of lysosomal induced tissue autolysis [19].

In this aspect the increased copper level associated with ulcer to endocrine disturbance, where growth hormone and insulin-like growth factor-1 decline with ulcer where [18] found that when the main serum binding protein-3 (IGFBP-3) that carries insulin-like growth factor-I increases, the free hormone decreases and results in body weight loss followed gastric ulceration [20].

Also, the recorded decreased serum calcium and phosphorus followed gastric ulceration which may be due to the suppressed appetite and off food followed the acute gastritis and gastric ulcerations [5]. Also these results might be related to the disturbed mineral absorption followed the gastric ulcerations [21].

The recorded significant increases in serum nitric oxide level throughout the experimental duration may be attributed to the oxidative stress which illustrated why level of NO was increased in the experimental group after complete induction of ulcers at zero days that was parallel with a decrease in SOD level in experimental group rats. These results were also in consistency with [22].

In this study the experimental groups clarify that NO level not returned down to it's the healthy level after 3 weeks of induction of ulceration ,this denotes that acutely ulcers could be mostly associated with multiple increases in the NO that can initiate continuously the inflammatory cascade of reactions till be terminated by therapy as reported by [23] found that NO levels in sera of patients with that were elevated could be reduced and the ulcers conditions suppressed in experimental animal after using the NO inhibitors.

Since gastric ulceration is characterized by an increase in NO production when compared with other infectious disease, it is possible that a gradient of NO production contribute to the increased appetite loss which is an early feature of gastric ulceration and leads to loss of body weight as proved by [24] who found that the increased NO does not only enhance loss of appetite but also may decrease pituitary GH mRNA expression as a result of further biosynthesis and release of hypothalamic somatostatin (IGF-1),that ends in progressive loss of body weight of animal with gastritis which could be attributed to the vasodilatation effects of nitric oxide.

The pathogenesis of gastritis and the inflammatory processes depend upon the increased level of NO in the area of the reaction. By virtue, maintenance of high levels of NO can induce cellular and biochemical changes [25].

The presented data in Table (2) showed that the experimental induced gastric ulceration followed by very high significant increase in serum prostaglandin E_2. This result may be contributes to the swelling of gastric mucosa and hyperpyrexia in subjects with gastric ulcer. It was also reported that PGE_2, plays a major role in tissue edema production in inflamed stomach in rat models it also may be due to the increased values of NO which plays a role in the initiation and progression of gastric membrane inflammation, increases vascular permeability, and enhances PGE_2 biosynthesis [26].

Comprehensively, several lines evidence suggest that at least some of the proinflammatory aspects of gastritis are mediated by PGs, specifically; PGE_2 that has been observed to associate edema and erosion of gastric mucosa commonly detected in patients with gastric ulcers [27] make it rational to accept the dramatic increase in NO levels of gastric ulcers untreated rats that have been detected in this study. Furthermore, [28] reported that IL-1 stimulates the production of NO and PGE_2 form the mediators that probably contribute to the tunica mucosa destruction that has been seen in gastric ulceration. Practically, these activated macrophages take up small debris particles and produce inflammatory mediators, prostaglandins, soluble adhesion molecules and growth factors [26]. Concomitantly, [29] found that in acute ulcerative inflammation the granulocytes and macrophages accumulate in the affected area and produce large amounts of superoxide anion and hydrogen peroxide radicals. Moreover, [30] observed that the polymorphonuclear leukocytes can ingest immune complexes, with the polymorphonuclear, with the resultant production of reactive oxygen metabolites and large amounts of prostaglandins and leukotrienes by cells in gastric ulceration.

The increased number of inflammatory cells accompanied with biochemical process produce lipopolysaccharide, interleukins, prostaglandins interferon-gamma, which called inflammatory mediators. These inflammatory mediators have a role as intracellular messengers to these inflammatory cells to produce number of calcium-independent nitrous oxide synthase [31]. So, the recorded increase in prostaglandins level followed gastric ulcer due to neutrophil accumulation oxidatively inactivates the antioxidant gastric peroxidase leading to increased oxidative damage to the mucosa and the neutrophils infiltration into gastric mucosa produce superoxide radical anion that in a chain reaction attach membrane lipids leading to their peroxidation to release malondialdehyde and 4-hydroxynonenal and closely relates to the focal lesions [32].

The recorded data table (2) showed that the experimental induction of acute gastric ulceration in rats accompanied by significant decrease in erythrocytes total superoxide dismutase activity (t-SOD) throughout the period of experiments on comparison with the mean values in control group. Oxygen free radicals stand in the back of pathogenesis of many degenerative diseases; causative or resultive still an enigma although evidence of antioxidants preventing gastric ulceration is accumulating [33], [34]. Imbalance between oxidant free radicals production and their peroxidative by-products and antioxidant defenses results in oxidative stress. It results in reduced glutathione depletion with shift in the redox states of thiol/disulfide redox couples, lipid peroxidation and

membrane damage, DNA and protein oxidation; and activation of proteases, nucleases and protein kinases as indirect manifestations [9]. Aspirin administration increased the concentration of MDA, an index of lipid peroxidation, in the gastric mucosa compared to the normal control and probiotic control groups. Our results support the importance of lipid peroxidation in the pathogenesis of aspirin induced gastric damage [35]. Induction of t-SOD by superoxide anion to decrease the dangerous effect of the free radical on cell membrane was predicted as a cellular respond to destructive free radicals, to inhibit the primary phase of the gastritis [36], [37]. However, effect of SOD was predicted to be decreased in secondary phase of gastric ulceration, this means that after phase of gastritis reaches to chronicity, the effect of this SOD enzyme could be diminished or exhausted where, as a chronicity of gastritis [24]. The present study chose to investigate oxidative stress rather than measurement of individual effectors and/or end markers of, e.g., t-SOD. The latter is inappropriately and repeatedly used as a damage marker despite is possible implication in production of cytoprotective PGE_2 as reported by this laboratory and other investigators particularly when lipid peroxidation is modest. The decrease in t-SOD is a potential destructive indicator to gastric mucosa and the body at large. The model ulcerated rats showed high gastric juice total peroxide content.

The recorded data in Tables (1 and 2) showed that there were non-significant changes in serum glucose, insulin, sodium and potassium followed the induction of acute gastric ulcerations.

V. Conclusion

From the recorded results it can be concluded that

- Oral administration of aspirin induce gastric lesion ulcer model.
- Increased PGE_2 and oxidative stress are important factors involved in the pathogenesis of gastric ulceration.
- Increased PGE_2 can be considered as a new trigger against gastric ulceration.

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