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GASTROPROTECTIVE EFFECTS OF FLAVONOIDS IN PLANT EXTRACTS

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The purpose of this paper is to overview the relations between plant-originated substances and their bioactivity measured in terms of antioxidant, cytoprotective and antiulcer activities. In addition, we assessed whether these compounds are capable of affecting the gastric mucosal lesions induced by absolute ethanol applied intragastrically (i.g.). The following plant-originated flavonoid substances were considered; Solon (Sophoradin extract), Amaranth seed extract, grapefruit-seed extract (GSE) and capsaicin (extract of chilly pepper). The area of gastric mucosa lesions and gastric blood flow were measured in rats with ethanol-induced lesions without (control) and with one of the tested substances without and with capsaicin denervation of afferent nerves or administration of L-nitro-arginine (L-NNA), an inhibitor of nitric oxide synthase (NOS). Male Wistar rats, weighing 180-220 g fasted for 24 h before the study where used 100% ethanol was applied i.g. to induce gastric lesions, whose area was determined by planimetry. Gastric blood flow was assessed using electrolytic regional blood flowmeter. All tested plant-originated substances afforded gastroprotection against ethanol-induced damage and this was accompanied by increase in gastric microcirculation, both changes being reversed by pretreatment with neurotoxic dose of capsaicin or by pretreatment with L-NNA. We conclude that plant-originated flavonoid substances are highly gastroprotective probably due to enhancement of the expression of constitutive NOS and release of NO and neuropeptides such as calcitonin gene related peptide (CGRP) released from sensory afferent nerves increasing gastric microcirculation.

Key words: *plant gastroprotection, Solon, capsaicin, grapefruit seed extract, Amaranth, L-NNA*

INTRODUCTION

Gastric mucosal layers play a role of a barrier that limits an exposure of the gastric mucosal cells to numerous injurious luminal agents and irritants of exogenous and endogenous origin. Mucosal surface epithelium is a subject of attack by physical, chemical or microbiological agents acting from the gastric lumen, which are involved in multiple pathologies, such as gastritis, peptic ulcer or gastric cancer. Pretreatment with different substances could effectively prevent gastric mucosa from the development of erosions and ulcerations. This action, called gastro- or cyto-protection is not related to the inhibition of gastric acid secretion and known to account for gastroprotection by various irritants and ulcerogens (1).

This article overviews the potential role and also some basic mechanisms of plant-originated gastroprotective flavonoid substances applied intragastrically (i.g.). Recent studies found that different substances from plant sources, not only afford gastroprotection but also accelerate ulcer healing. They may also possess anti-inflammatory action by suppressing the neutrophil/cytokine cascade in gastrointestinal tract (GIT) (2), promoting tissue repair though expression of various growth factors (3), exhibiting antioxidant activity (3), scavenging reactive oxygen species (ROS) (4, 5), showing anti-nucleolytic, cytochome P450 2F1 inhibitory acitivity, anti-necrotic and anti-carcinogenic activities (6, 7).

MATERIAL AND METHODS

Wistar male rats weighing 200-220 g were used in studies with production of acute gastric lesions and measurement of gastric blood flow. The study was performed according to Helsinki declaration and met approval from the Ethical Committee of Jagiellonian University College of Medicine. All animals were fasted 24 h before the study but had free access to water. They were placed in individual Bollman's cages to prevent coprophagy.

Acute gastric lesions were induced by intragastric (i.g.) administration of 100% ethanol in a volume of 1 ml using special oro-gastric metal tube as described previously (8-10). One hour after ethanol application, the animals were lightly anesthetized with ether and then after midline incision, the stomach was approached using electrolytic regional H_2-gas clearance technique employing Electrolytic Blood Flow Meter (Biomedical Science Co., Tokyo, Japan) with single electrode both for the generation of H_2 and measurement of its concentration as described before (9). The tested plant-originated preparations were administered in various doses 30 min before ethanol application and in case of L-NNA administration, this agent was injected intravenously (i.v.) at various doses about 30 min before application of plant-originated gastroprotector. Then, animals were killed by blow to the head and the stomach was removed, opened alone the greater curvature and the area of gastric lesions was measured by planimetry (Morphomat, Carl Zeiss, Berlin, Germany. The area of lesions in each animal was expressed in mm^2. Several groups of animals, each consisting of 6-8 animals were used.

Mean values were calculated for the area of gastric lesions and the gastric blood flow. The results were analyzed by Student's t test, and P values less than 0.05 were considered significant. All results are given as means ± standard error of mean (S.E.M.).

RESULTS AND DISCUSSION

Plant-originated substances, exhibiting gastroprotective action against ethanol-induced gastric lesions

There are various plant-originated "gastroprotectors" with different composition that have been used in clinical and folk medicine for many countries due to their beneficial effects on the mucosa of GIT. In China and Japan, polyphenol extracts such as Sopharadin extracts, containing flavonoids and its synthetic flavonoid derivative known as Solon are widely employed in peptic ulcer therapy and also as food additives and nutritional supplements, mainly because of their strong inhibition of prostaglandin (PG) metabolism and vasoconstrictive leukotriene inhibition (7). Our previous studies (8, 9) with that agent demonstrated that it enhances healing of chronic gastric and duodenal ulcers induced by acetic acid and that it acts probably through the increase in mucosal PG content probably due to inhibition of 15-OH-PG dehydrogenase, a PG hydrolyzing enzyme. This agent is of special interest as it is widely used in Japan, where the rate of peptic ulcer disease is still higher than in Europe, as gastric protective and anti-ulcer agent employed in combination with classic antiulcer drugs such as H_2-receptor antagonists or proton pump inhibitors.

In this study, Solon (Taisho Pharmaceutical Co, Tokyo, Japan) given topically (i.g.) resulted in dose-dependent protection against acute gastric lesions produced by 100% ethanol, the corrosive substance for the gastric mucosa (*Fig. 1*). The gastroprotection by Solon was accompanied by dose-dependent increase in gastric mucosal blood flow. Both the gastroprotection and gastric hyperemia can be attenuated by the pretreatment with unspecific NOS inhibitor such as L-NNA, indicating that local release of NO, possibly due to increased expression and activity of constitutive and/or inducible NOS plays a major role in Solon-induced gastroprotection. The major compounds responsible for the gastroprotection by Sophoradin derivatives are probably various flavonoids, the biologically active agents with high pharmacological and vasoactive potency (10).

Therapeutic effects of Amaranth seeds, that were related to scavenging of endogenous ROS , could account to the reported maintenance of liver integrity and homeostasis (11), but no attempts were made to determine whether this plant exerts any gastroprotective activity. In accordance to our experience, Amaranth results in gastroprotection against 100% ethanol and its favorable effect could be reversed by the pretreatment with neurotoxic dose of capsaicin that is known to cause functional ablation of sensory afferent nerves and release of gastroprotective sensory neuropeptides such as calcitonin-gene related peptide (CGRP) (*Fig. 2*). Based on the results we can speculate that Amaranth acts on gastric mucosa to stimulate afferent nerves and increase in gastric microcirculation, but further studies are required to examine the protection by amaranth in depth.

Grapefruit-seed extract (GSE), containing flavonoids, has been shown to possess antibacterial, antiviral and antifungal properties (12,13). This beneficial

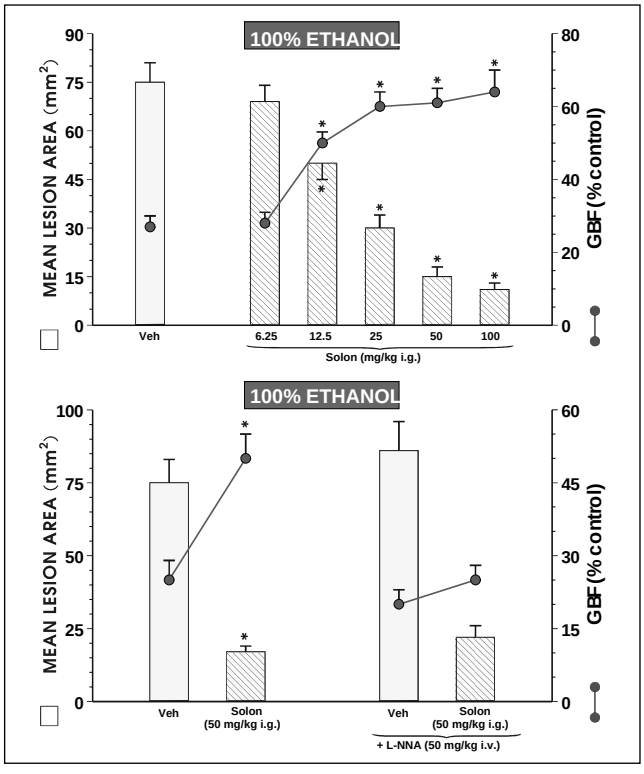

Fig. 1. Mean area of 100% ethanol-induced gastric lesions and the gastric blood flow (expressed as percent of control) in rats without (vehicle only) and with pretreatment with graded doses of Solon (upper panel). The effect of L-NNA combined with Solon (50 mg/kg i.g.) on mean lesion area and the accompanying changes in the GBF. Each column is a mean (± SEM) of 5-8 rats. Asterisk indicates significant ($P < 0.05$) change as compared to the vehicle (control) values. Cross indicates a significant change as compared to the values in Solon treated animals without L-NNA.

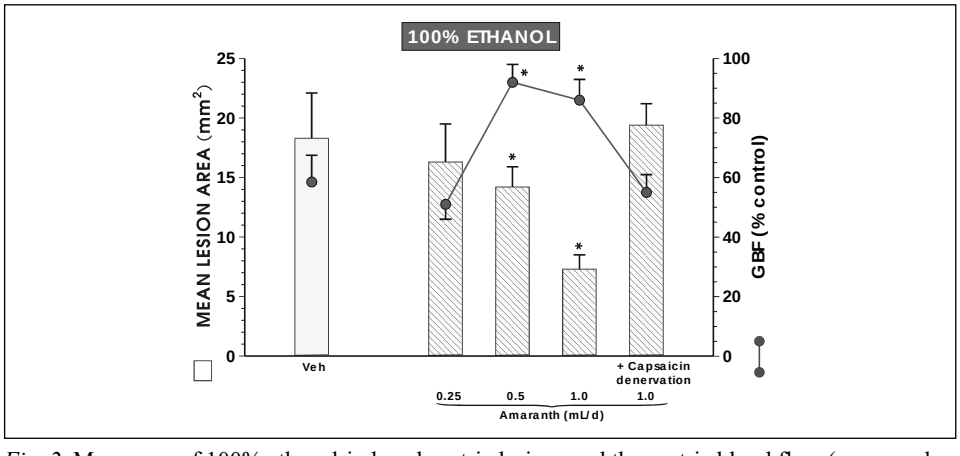

Fig. 2. Mean area of 100% ethanol-induced gastric lesions and the gastric blood flow (expressed as percent of control) in rats without (vehicle only) and with pretreatment with graded doses of Amaranth (extract of Amaranth seeds) without or with sensory denervation by neurotoxic dose of capsaicin (125 mg of capsaicin injected s.c. 2 weeks before experiment). Each column is a mean (± SEM) of 5-8 rats. Asterisk indicates significant ($P < 0.05$) change as compared to the vehicle (control) values. Cross indicates a significant change as compared to the values obtained in animals without capsaicin denervation.

action of GSE was attributed to the antioxidative activity of citrus flavonoids found in grapefruit (14,15) such as naringenin because this major flavonoid found in the grapefruit juice, exhibited the potent antibacterial and anti-*Helicobacter pylori* activity *in vitro* (13, 15) and was also recently implicated in cytoprotection against injury induced by algal toxins in isolated hepatocytes (16). Moreover, naringenin, the bioactive component of GSE, showed gastroprotective activity due to increase expression of prostaglandins biosynthesis (17). Furthermore, it was shown to exhibit anti-cancer activity against human breast cancers (18). Therapeutic efficacy of citrus fruits such as red grapes and grapefruits is emphasized by the fact that they contain different classes of polyphenolic flavonoids, that were shown to inhibit platelet aggregation thus decreasing the risk of coronary thrombosis and myocardial infarction (19).

The involvement of flavonoids present in grapefruit seed extract in the mechanism of gastric mucosal defense has been little studied. Our present study with GSE (Citro, HERB-PHARMA, spol s.r.o., Welke Ludince, Slovakia) confirmed that *in vitro* GSE is highly antibacterial and antifungal agent. Most important, we found that this extract in minute doses causes dose-dependent diminution of acute gastric lesions induced in rats by 100% ethanol. The mechanism of this protection appears to be dependent on the functional activity of sensory nerves releasing CGRP because; a) capsaicin-deactivation of these nerves markedly reduced the protection and increased gastric circulation provoked by GSE and b) exogenous CGRP, administered in physiological dose to replenish the deficiency of this peptide caused by capsaicin, restored the protective efficacy of GSE. No study so far has been undertaken to examine the gastroprotective or ulcer healing efficacy of GSE, but the fact that it is a potent anti-*H. pylori* substance and exerts profound gastroprotection in laboratory animals suggests that it might be also effective in humans with *H. pylori*-induced gastritis. The Citro containing GSE is available in Poland and Slovakia without prescription and its application in the doses used in this study, does not cause any side effects nor any inhibition of gastric acid secretion. (*Fig. 3*).

In another report, naringenin, a major GSE flavonoid, has been just reported to exhibit gastroprotection against the gastric injury induced by absolute ethanol predominantly due to the increase in the mucus secretion (17). It is of interest that this gastroprotective effect of *naringenin* and accompanying increase in the mucus secretion, were, in part, attenuated by indomethacin suggesting the involvement of endogenous PG in the mechanism of this flavonoid-induced gastroprotection.

Our group demonstrated long time ago that meciadanol, a synthetic flavonoid, similar to catechin flavonoid, that inhibits histidine decarboxylase and decreases histamine content in the stomach, attenuated gastric mucosal lesions produced by ethanol and aspirin *via* mechanism independent of gastric acid secretion and endogenous prostaglandins (PG) (20).

One of the most interesting substances that has been obtained from chilly peppers and present in spicy plants such as ginger or black pepper is capsaicin

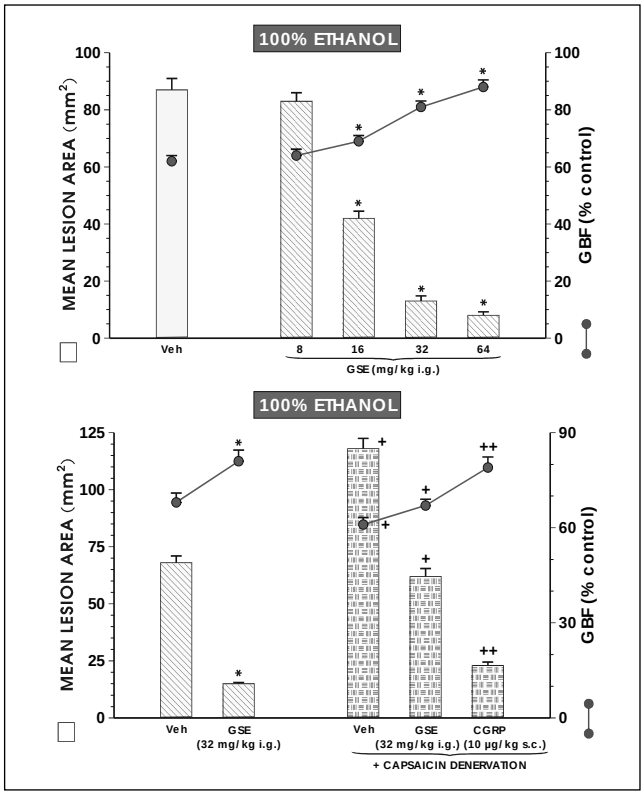

Fig. 3. Mean area of 100% ethanol-induced gastric lesions and the gastric blood flow (expressed as percent of control) in rats without (vehicle only) and with pretreatment with graded doses of grapefruit seed extract (GSE) (upper panel) and effects of capsaicin denervation without or with addition of calcitonin-gene related peptide (CGRP) (lower panel). Each column is a mean (± SEM) of 5-8 rats. Asterisk indicates significant ($P < 0.05$) change as compared to the vehicle (control) values. Cross indicates a significant change as compared to the values in rats without capsaicin denervation. Double cross indicates a significant change as compared to the values obtained in capsaicin denervated rats without CGRP administration.

(21-24). This substance acts on sensory neurons to stimulate their membrane receptors, predominantly vaniloid (VR)-1 receptors, and release various kinins such as CGRP and substance P. When applied in large dose capsaicin destroys selectively C-fiber neuronal endings leading to inactivation of sensory nerves and the loss of all reflexes in which these nerves are involved. In smaller dose, capsaicin is the potent gastroprotective agent and stimulant of gastric microcirculation. According to our experience (*Fig. 4*), capsaicin given i.g. in doses ranging from 0.1 to 0.5 mg/kg reduced dose-dependently the ethanol-induced acute gastric hemorrhagic lesions and this is accompanied by dose-dependent increase in gastric microcirculation. These effects can be abolished, also dose-dependently, by the pretreatment with L-NNA that reverses also the gastric hyperemic effects of capsaicin. Capsaicin is ineffective in rats with deactivated sensory nerves by large dose of capsaicin (125 mg sc two weeks before the experiment). Clinical usefulness of capsaicin has not been so far demonstrated in humans but it is an excellent tool to study the implication of sensory nerves in the gastroprotection induced by various gastroprotective agents including GSE, Amaranth and various gastroprotective drugs and substances.

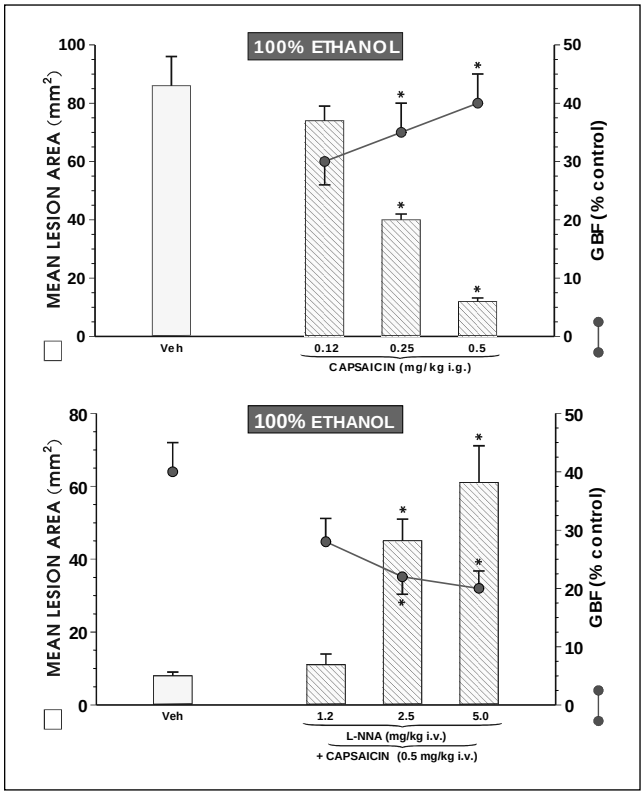

Fig. 4. Mean area of 100% ethanol-induced gastric lesions and the gastric blood flow (expressed as percent of control) in rats without (vehicle only) and with pretreatment with graded doses of capsaicin (upper panel) without and with addition of L-NNA, a NO synthase inhibitor (lower panel). Each column is a mean (± SEM) of 5-8 rats. Asterisk indicates significant ($P < 0.05$) change as compared to the vehicle (control) values.

In India, an important gastroprotective effect was shown in studies on several experimental ulcer models in rats using aqueous extract of *Neem (Aradirchta indica)* bark (25), ethanol extract of *Aqeratum conyzoides* (26), *Bacopa monniera* and *Azadirachla indica* extract (27). In Mexico the root bark of Hippocratea excelsa, locally known as "Cancerina"(28) and Azadirachta indica extract (29) are used in gastric disorders. In Spain, the flavonoid fraction (ethylacetate extract) of the plant *Erica andevalensis Cabezudo-Rivera* (30) and the plant ternatin, a tetramethoxyflavone, isolated from *Eglets viscose,* are used because of their gastroprotective properties, probably unrelated to endogenous release of gastroprotective prostaglandins (PG) (31).

In Chinese medicine *Phellodendri cortex (Phellodendron amurense)* Ruprecht has been used to treat the patients who suffer from gastroenteritis, abdominal pain or diarrhea, because the berberine-free fraction of the extract from this plant has anti-inflammatory activity and additive effect on the cytoprotection and reduction of gastric acid secretion (32).

Grape seeds (GSP) and skins are good sources of phytochemicals such as gallic acid, catechin and epicatechin known to exert scavenging of ROS (33-37). Data accumulated have demonstrated that this extract may serve as a potential

therapeutic tool in cardioprotection *via* a number of novel molecular mechanisms (39,40). GSP improve insulin sensitivity and/or ameliorate ROS formation and reduce the sings/symptoms of chronic age-related disorders including syndrome X (41). Identification these components has been interpreted on molecular basis of "French Paradox" in which good red wine is beneficial for the cardiovascular system (42).

The influence of black currant juice on lowering effect on protein and lipid oxidation was similar in magnitude to the known liver protecting agent kolaviron (43,44). The results of investigations on modulatory effects of kolaviron, a

Table 1. Summary of the sources of plant cytoprotectors and their known physiological actions on GIT.

Physiological actions	Origins
Gastroprotective and antiulcer	Grapefruit (*Citrus paradisi*) seeds *Panax ginseng*
Induced changes in amount and glycoprotein content of gastric mucus	*Erica andevalensis Cabezudo-Rivera* UL-409, herbal formulation
Preventive and curative effects	Sea buckthorn (*Hippophae rhammoides L.*)
Inhibition the basal and histamine-induced gastric acid secretion	*Azadirachta indica* Chinese cinnamon *Phellodendron amurense* Ruprecht
NO-induced rise in mucosal blood flow	*Gingi biloba* *Silybum marianum* Grapefruit seeds *Bacopa monniera* Grape seeds
Mucus and alkaline secretion	*Tasmannia lanceolata* *Bacopa monniera* *Azadirachta indica* *Mikania cordata* *Solon (Sophoradin)*
Prostaglandin release	*Tamannia lanceolata* *Petasites hybridus* *Ruta chalepensis L. (Rutaceae)*
Hepatoprotective	*Tinospora bakis (Menisoermaceae)* *Premma tomentosa (L. Verbanacae)*
Anticancerogenic	Grapefruit seeds *Garsinia kola* Grape seeds

biflavonoid from *Gacinia kola*, natural antioxidant, on drug-induced kidney toxicity show influence on the cellular redox status and depression of membrane protein activities and may be revalent in the chemoprevention of oxidant-induced genotoxicity and possibly human carcinogenesis (43).

Gingo biloba extract can improve memory, prevents pancreatitis and enhances gastroprotection against necrotizing agents and accelerates healing in rats with duodenal ulcer through cytoprotective and antioxidant actions (45,46).

Extract from *Petasites hybridus* with three main compounds oxopetasan esters, petasin and isopetasin inhibits the biosyntesis of the vasoconstrictive peptide leukotrienes, this contributing to gastroprotection and spasmolytic activity (47).

Table 2. Active constituents in plant gastroprotectors. The presented data based on the reviewed references cited in the text

Active substances	Sources
Polysacchrides	*Opunta ficus indica cladodes*
Flavonoids	*Erica andevalensis Cabezudo-Rivera* *Garcinia kola seeds (*kolaviron*)* *Achyrocline satureioides* *Silydum marianum* *Scutellaria baicalensis*
Proanthocyanidins	*Grape seeds extract* *Grape skin extract*
Polyphenolic natural compound	*Neem (Azadiracta indica)* *Curcumin*
Diterpnes	*Tasmannia Lanceolata* *Egletes viscosa*
Sesquiterpenes	*Petasites hybridus* *Emblica officinalis*
Saponins	*Hippocrata excelsa*
Scvalen	*Amaranth*
Sitosterol	*Egletes viscosaless*
Ternatin	*Horse chestnuts*
Escins	*Cinnamonum cassia*
Berberine	*Phellodendron amureuse Ruprecht*

CONCLUSIONS

1. We demonstrated that plant-originated substances such as Solon - Sophoradin root originated flavonoid, seed extract of Amaranth, extract of grapefruit seeds (GSE) - Citro, and capsaicin present in chilly pepper, all exerted beneficial and dose-dependent reduction in acute gastric lesions induced by corrosive concentration (100%) of ethanol and this reduction was accompanied by dose-dependent rise in gastric blood flow;

2. The mechanism of this protection is closely related to the increase in gastric microcirculation probably caused by stimulation of afferent nerves and release of NO in the mucosa because the protection and gastric hyperemia could be significantly attenuated following the inactivation of sensory afferents by neurotoxic dose of capsaicin and the application of NOS inhibitor, L-NNA;

3. This study provides an evidence that extracts originating from the plants used in ancient herbal medicine appear to contain highly effective, but little studied in humans compounds, most likely flavonoids, that are capable of protecting the gastric mucosa from necrotizing substances and possible useful in the therapy of acute and chronic gastric ulcerations. Among plant antiulcer drugs, only Solon is now widely used in gastritis and peptic ulcer therapy in certain countries.

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