A composition for use in the prevention and/or treatment of gastric or gastroesophageal diseases

The present invention relates to a composition of substances preferably obtained from natural sources, which is effective in the prevention and/or treatment of gastric or gastroesophageal diseases.

More in particular, the present invention relates to a composition of substances preferably obtained from natural sources, which is effective in the prevention and/or treatment of gastric or gastroesophageal diseases selected from the group consisting of heartburn, dyspepsia, and gastroesophageal reflux disease.

Heartburn, also referred to as retrosternal pain, is a painful symptom generally located near the sternum and associated with gastrointestinal disorders, in particular stomach disorders.

Heartburn can arise spontaneously or following ingestion of irritating foods or particular drugs. It is generally associated with reflux, which brings gastric or duodenal material in contact with a dysfunctional esophagus. In the absence of a diagnosis of hiatal hernia or aerophagia, heartburn is to be considered as a functional disorder. If heartburn is secondary to diseases such as reflux oesophagitis, it usually appears daily and any factor capable of facilitating reflux, such as leaning forward, causes the appearance of the disorder. Hindered gastric emptying and nocturnal decubitus can increase heartburn.

Since the main cause of heartburn is the contact of the acid with the esophageal mucosa, one of the most widely used strategies for the treatment of this disorder is the use of drugs capable of reducing acid secretion in the stomach through different mechanisms of action.

Gastric secretion is a process dependent on neuronal (acetylcholine), paracrine (histamine), and endocrine (gastrin) factors. These factors act on the receptors M_3, H_2, CCK_2, respectively, mainly located on the membrane of gastric parietal cells.

Three main endogenous compounds are responsible for gastric secretion and each plays a

specific role in adjusting the amount of secretion to functional requirements:

- GASTRIN: A protective hormone secreted by G cells of the gastric antrum; it is the strongest stimulator of gastric acid secretion. The gastrin-releasing peptide released by enteric terminals is among the stimuli that cause the release thereof. Gastrin acts on parietal cells, chief cells, and enterochromaffin-like cells, which are responsible for histamine secretion.
- HISTAMINE: It binds to the histaminergic receptors of parietal cells. Histamine, by interacting with the H2 receptor, in synergy with gastrin stimulates the release of hydrochloric acid and pepsin.
- ACETYLCHOLINE: It stimulates parietal cells and chief cells, thus increasing the secretory activity of the stomach, and also increases smooth muscle contraction.

One of the mechanisms involved in the inhibition of gastric secretion is the action of somatostatin secreted by D cells of the gastric antrum, which inhibits gastric secretion. Its release is induced by a gastric intraluminal pH of less than 3, which simultaneously suppresses gastric secretion through a negative feedback mechanism.

Acid secretion by the stomach takes place through the action of the H^+/K^+- ATPase proton pump, which is present in parietal cells and activated by the cyclic AMP and calcium ion route.

The most widely used pharmacological treatments in case of gastric hyperacidity are the following:

- Inhibitors of the proton pump. After being absorbed, they penetrate the gastric parietal cells and covalently bind to the proton pump (sulphydryl groups), thereby irreversibly inactivating it. Prolonged use of these drugs is associated with increased cardiovascular risk and increased risk of experiencing chronic renal failure.
- Antagonists of the H_2 receptor. They reversibly compete with histamine for binding to H_2 receptors. They are definitely less powerful than the pump inhibitors.
- Prostaglandin analogues. They induce a similar effect as that generated by prostaglandins through interaction with EP_3 receptors, reduce intracellular cyclic AMP, and consequently gastric acid secretion. Furthermore, PGE_2 exerts cytoprotective effects,

including mucus and bicarbonate secretion, in addition to increased mucosal blood flow by promoting NO production by NOSs with consequent vasodilation.

The term dyspepsia refers to a series of episodic or persistent symptoms, mainly perceived at the upper part of the abdomen. Dyspepsia can be associated with a sense of heaviness and anorexia.

This is a rather common gastrointestinal disorder, which is found in most of the world's population: in the United Kingdom, over 40% of adults reported at least one episode of dyspepsia in the last 12 months.

Dyspepsia is classified as follows:

- Functional or idiopathic dyspepsia. A disorder of the upper part of the abdomen, which could not be linked to an adequate organic, biochemical or structural cause.
- Organic or secondary dyspepsia. It may have several causes, including diseases such as esophagitis, gastritis, duodenitis, pancreatitis, hepatitis, but also food poisoning, use of drugs or certain types of medicaments.

There are several pharmacological treatments for this disorder. The main ones are as follows:

- Prokinetic-acting drugs. They act through several pharmacological mechanisms of action. They lead to an increase in the frequency of movement of the stomach, thereby increasing the gastric emptying rate.
- Inhibitors of the proton pump. As previously described, they inhibit gastric secretion.
- Antacids. They react with the hydrochloric acid present in the stomach, increasing the pH of the stomach contents and preventing damage to the esophageal mucosa in case of reflux.
- Bile acids;
- Anxiolytics.

Gastroesophageal reflux refers to unintentional and unconscious passage of part of the stomach contents into the esophagus, with no participation of the stomach and abdomen

muscles.

The esophagus is a 25-30 cm long canal, which connects the mouth with the stomach; two sphincter structures can be identified along its length: the first between the hypopharynx and the cervical portion of the esophagus (Upper Esophageal Sphincter, UES), the second, i.e. the Lower Esophageal Sphincter (LES), at the esophagus-stomach junction. The latter is a high-pressure area representing the main anti-reflux structure, due to its location between the negative-pressure intra-thoracic area and the positive-pressure intra-abdominal area. Therefore, under normal conditions, an increase in abdominal pressure has repercussions on the LES, thus preventing the ingested material from going back into the esophagus. Under physiological conditions, the LES is closed and relaxes for a period of about 3-10 seconds after swallowing. Further anatomical structures, in addition to LES, which contribute to the maintenance of the anti-reflux barrier are:

- The angle of His, the acute angle formed between the esophagus and the gastric fundus;
- The phrenoesophageal ligament;
- The diaphragm collar consisting of diaphragm bundles, which, by placing itself like a scarf around the esophagus, squeezes the lumen thereof during the inspiratory phase.

Several factors are included in the pathogenesis of the gastroesophageal reflux disease (GERD), such as for example:

1. Deficiency of the lower esophageal sphincter anti-reflux barrier, which serves to mechanically prevent the gastric juices from refluxing into the esophagus.
2. Delayed gastric emptying due to anatomic abnormalities or functional alterations: (i) anatomic abnormalities: stenosis of the pylorus (the terminal region of the stomach, which regulates the passage of gastric contents into the duodenum); (ii) functional alterations: motor alterations of the fundus (the region responsible for emptying liquids)
3. Deficiency of the esophageal clearance mechanism, which has the purpose of minimising contact between the esophageal mucosa and gastric juices by acting both through esophageal peristalsis and acidic residue neutralisation by the saliva.
4. Gastric hyperacidity.
5. Aggressiveness of the gastric contents that flow back into the esophagus, due the

action of hydrochloric acid.

6. Duodenogastric reflux with pancreatic and biliary secretions passing into the stomach, which in the case of gastroesophageal reflux can result in more severe lesions.

Other predisposing factors include smoking, improper dietary and behavioural habits (generous meals, high-fat foods, caffeine); drugs, pregnancy and obesity may also exacerbate GERD. A hiatal hernia (the extension of a portion of the stomach inside the thorax, through a hole in the diaphragm called the esophageal hiatus) also frequently accompanies GERD and may contribute to prolonged exposure to gastroduodenal contents. Generally, the walls of the esophageal hiatus adhere closely to the esophagus, but it can happen that the anchoring structures of the lower portion of the esophagus lose tone, thus favouring the ascent of a small part of the stomach into the thorax.

In any case, whatever the cause may be, the frequent and repeated contact of the regurgitated gastric material with the esophageal mucosa exerts thereon a damaging action that is all the more serious the longer the contact time and the lower the pH of the reflux. Over time, the persistent phlogistic action affecting the esophageal mucosa leads to an inflammatory reaction that can evolve into ulcerations, stenosis and so-called columnar metaplasia (or Barrett's epithelium, the single most important risk factor for the development of esophageal adenocarcinoma). Symptoms considered to be typical are retrosternal heartburn (defined by the patient as a burning sensation that starts at the stomach or the lower portion of the thorax and rises towards the neck) and regurgitation (perception of acidic and bitter tasting liquid inside the oral cavity), symptoms whose specificity for GERD is equal to 89 and 95%, respectively. Frequent albeit less specific symptoms are odynophagia, dysphagia, belching, epigastric pain, bloating, and digestive difficulties. Some of these symptoms characterise the diagnosis of functional dyspepsia and it is known that between 10% and 17% of patients requiring medical intervention for dyspepsia suffer from GERD.

GERD is one of the most common pathological conditions encountered by gastroenterologists. A study on the prevalence of the disease showed that GERD has a prevalence of 10-20% in Western countries versus only 5% found in Asia; in particular, a

greater number of cases were found in North America, then in Northern Europe, and in Southern Europe.

Scientific studies show that the symptoms of the disease have a strong impact on the quality of life as the persistent reflux symptoms, even during treatment with proton pump inhibitors, are associated with reduced physical and mental wellbeing.

Since it is a chronic disease, conventional treatment is almost always long lasting and consists, according to severity, in lifestyle changes (eliminating chocolate, caffeine, alcohol, cigarette smoking, losing weight etc.), pharmacological treatments, and surgical therapy.

Drug classes commonly used for treating GERD include antacid drugs, histamine H_2 receptor antagonists, proton pump inhibitors (PPIs), and prokinetic agents.

Antacids are over-the-counter drugs that offer rapid relief to the symptoms of the disease, but are not able to induce a curative effect in erosive esophagitis. These drugs contain carbonates or bicarbonates or other basic salts that reduce the acidity of the stomach by reacting with hydrochloric acid and releasing carbon dioxide.

H_2 antagonist drugs - such as ranitidine, famotidine, cimetidine - provide temporary relief of symptoms, although with a slower onset time than antacids. Use for prolonged periods of time is not recommended as patients may develop tolerance within 1-2 weeks, and in any case the effect of these drugs is not curative.

PPI drugs (pantoprazole, lansoprazole, omeprazole etc.) represent standard treatment in gastroesophageal reflux disease, in fact the number of prescriptions for these drugs has doubled in the last 10 years. These prescriptions are often associated with those of steroidal or non-steroidal anti-inflammatory drugs. The mechanism of action of PPIs includes proton pump blockage at the parietal cells of the stomach; this hydrogen/potassium ATPase pump causes the release of hydrochloric acid into the stomach lumen. Compared to H_2-antagonists, these drugs act more rapidly and above all have a healing effect on esophageal

injury. The most common side effects encountered in the treatment with PPIs are nausea, diarrhea, headache, insomnia, and anaphylactic reactions.

Prokinetic agents such as cisapride or metoclopramide activate the serotonin or dopamine receptors capable of increasing esophageal or gastric peristalsis. These drugs have a slow onset of action, short duration and no curative effect on the disease. They also exhibit several side effects such as tremors, dyskinesia, fatigue, and increased adverse events at the cardiac level, so their use is quite limited in the treatment of GERD.

In addition to the classic pharmacological remedies, alginates are also used for the symptomatic treatment of GERD. Alginates, such as sodium alginate, are natural polysaccharides, which upon contact with the gastric environment precipitate forming a low-density gel in minutes. Bicarbonates and carbonates, almost always present in commercially available alginate formulations, release carbon dioxide, which is trapped inside the alginate gel that is able to float on the gastric contents. The alginate gel is formed in the portion of the stomach close to the gastroesophageal junction, right where the acid pocket develops. In this way, the ascent of acid from the stomach to the esophageal canal is blocked or greatly reduced.

However, there is a continuing need to provide alternative treatments to the existing ones, which are effective in the prevention and/or treatment of gastric or gastroesophageal diseases, particularly heartburn, dyspepsia, and/or gastroesophageal reflux disease, but do not have the side effects and/or disadvantages of the state-of-the art treatments.

These and other needs are met by the present invention which provides a composition characterised in that it comprises a synergistic combination of active substances obtained from natural sources, the aforesaid combination having proved particularly effective against the aforementioned gastric or gastroesophageal diseases.

The composition of the invention is as defined in appended claim 1. Further features and advantages of the invention are defined in the dependent claims. The claims form an integral part of the present specification.

A detailed description of some preferred embodiments of the invention is provided hereinafter.

The synergistic composition of the present invention is a supplement useful for the treatment and prevention of gastric and gastroesophageal diseases, preferably heartburn, dyspepsia, and/or gastroesophageal reflux disease. In the composition of the present invention, the synergistic action takes place between the antacid, the extract from a plant of the genus *Musa*, and the protease enzyme.

The antacid present in the composition of the invention is any chemical compound commonly known as an effective antacid, i.e. a compound that is capable of reacting with the hydrochloric acid present in the gastric acids, thus raising the pH of the stomach, thereby reducing potential damage to the gastric and esophageal mucosa that could result from contact with the acid. The antacid is preferably a pharmaceutically acceptable basic salt, more preferably selected from the group consisting of carbonates, bicarbonates, citrates, hydroxides, metal oxides, trisilicates and any combination thereof.

The genus *Musa* includes plants from which the banana fruit is obtained, such as for example *Musa paradisiaca* and *Musa sapientum*.

Musa paradisiaca is a herbaceous plant up to 9 metres long with oblong fruits about 5-7 cm long or longer. *Musa sapientum* is a perennial herb that grows up to between 5 and 9 metres in height, with large inflorescences and sweet ripe fruits that have a thicker skin than other types of bananas.

There are about 300 banana varieties growing all over the world, but mainly in Asia, Indo-Malaysia, Australia and tropical and sub-tropical countries. India, the Philippines, China, Ecuador and Brazil are among the main producing countries.

Since ancient times, the banana was used in common practice to counteract several pathological conditions such as: diarrhea, dysentery, intestinal injury arising from ulcerative colitis, diabetes, uraemia, nephritis, hypertension, heart disease, etc. The many

features of this fruit are due to the different components that have been isolated.

Catecholamines such as norepinephrine, dopamine, serotonin, tryptophan and pectin were isolated from the pulp and the peel of *M. sapientum* and *M. paradisiaca* fruits. Flavonoids and derivatives (leucocyanidin, quercetin), sitosterol, sitoindosides, triterpenes, celluloses, hemicelluloses, amino acids, etc. were also found.

Among the various therapeutic activities boasted by the banana, its use in the treatment of gastric diseases such as ulcers is particularly interesting. Several scientific studies have shown that various components could be involved in the mucoprotective and antiulcerogenic activities. Pectin and phosphatidylcholine could bind the gastric mucosa, protecting its integrity. Furthermore, leucocyanidin has been considered by some authors as a mucosal protectant in different models of ulcer induced by aspirin, indomethacin, phenylbutazone, prednisolone, histamine, and others.

For example, in an ulcer model induced in rats, oral intake of the *M. sapientum* fruit extract (100 mg/Kg), due to the presence of leucocyanidin, resulted in protection of the mucosa through stimulation of proliferation and mucus secretion, and increased resistance of the gastric mucosa.

Another study carried out in 2013 assessed the action of the methanol extract from *M. sapientum* fruits on an aspirin-induced ulcer model. The results show the effectiveness of the extract at a dose of 100 mg/Kg comparable to that of ranitidine in protecting mucosal integrity.

Extracts from *M. sapientum* fruits, thanks to the presence of several compounds, mainly including the flavonoid leucocyanidin derivative and pectin, could be used as adjuvants in the treatment of diseases associated with acid hypersecretion or in case of poor digestion for the protection of gastric mucosal integrity.

The digestive enzyme in the composition of the present invention is a protease enzyme capable of breaking the peptide bonds in proteins, thus reducing them into peptides that

can be absorbed by the body.

Proteases suitable for use in the composition of the present invention are for example actinidain (present in kiwis), bromelain (present in pineapples), and papain (present in papayas).

The kiwi fruit is known for its various pharmacological properties, for its high content of vitamin C and, in particular, for the protease enzyme actinidain, which has been shown to be capable of promoting digéstion and gastric emptying.

Actinidain belongs to the cysteine protease family and contains free sulphydryl groups, which are essential for its activity. Major features of this enzyme include a wide pH range useful for catalytic activities and good stability at high concentrations; however, the enzyme is susceptible to oxidation.

Although its function is not yet fully clarified, actinidain was suggested to be involved in the defence of the kiwi fruit from pathogens. Numerous studies have shown that the rate and extent of digestion of proteins taken in with the diet have the ability to change the stomach emptying rate (SER).

Actinidain has also been shown to be able to degrade different types of proteins including beef muscle proteins, collagen, casein, and increase the digestive effect of pepsin at the gastric level.

An increase in gastric digestion of proteins from various sources (soy and beef protein, gelatin and gluten) has also been shown following administration of actinidain extracted from kiwi.

The effects of actinidain were also tested in rats, in particular in relation to gastric emptying. To do this, the gastric digestion of total dietary protein was quantified based on the analysis of free amino groups present in the chyme. In this case too, actinidain was shown to promote the digestion of beef muscle, gluten and soy proteins, but not gelatin and

milk proteins, due to their different structure. In addition, actinidain taken in with the diet is able to increase the gastric digestion of high molecular weight proteins. An increase in gastric emptying has been found to occur with diets containing beef muscle and zein, while this effect is not observed with diets based on gluten, gelatin, and soy and milk proteins. These findings have also been confirmed in pigs.

The literature suggests the use of 200 g of lyophilized kiwi fruit associated with 14 mg of purified actinidain containing 2154 U/g.

Within the scope of the present invention, the use of actinidain promotes protein digestion and consequently increases the stomach emptying rate: this reduces the frequency of reflux episodes, with consequent reduction of the damage to the esophageal mucosa.

Papain is obtained from the fruit of the *Carica papaya*, which belongs to the small Caricaceae family. The fruit, the leaves, and the latex extracted from the fruit are widely used because of their various pharmacological properties. The main chemical compound extracted from the fruit is papain, a proteolytic enzyme used as an active ingredient and as a reagent in the food and leather industry. In addition to papain, there are several other chemical compounds in the *C. papaya* extract, such as vitamins, proteins, fibres, carbohydrates, minerals, carpaine, carposide, and the enzyme myrosine.

Papain is a cysteine protease and belongs to a protein family provided with various activities including the endopeptidase, aminopeptidase, and dipeptidyl peptidase activities.

Three amino acid residues, Cys-25, Asn-175 and His-159, constituting the catalytic triad, are present within the active site of the enzyme. Asn-175 changes the orientation of the imidazole ring of His-159, which deprotonates the cysteine Cys-25; this allows the formation of a powerful nucleophile that is able to attack the peptide bond and form a thioether intermediate, which in turn is attacked by a water molecule that completes the hydrolysis reaction.

Papain does not have high selectivity of action, although hydrolysis catalysed by this

enzyme preferentially occurs at a peptide bond between a hydrophobic amino acid residue (Ala, Val, Leu, Ile, Phe, Trp, Tyr) and an arginine or lysine residue.

The use of this enzyme in the composition of the invention is based on its ability to hydrolyze the peptide bonds of proteins taken in with the diet. This allows a greater digestion rate and consequently a greater stomach emptying rate. Although there are no studies in the literature on the increase in stomach emptying rate following the administration of papain, there are several studies showing the same activity by actinidain, another cysteine protease isolated from kiwi. Given the similarity of the two enzymes, it is expected that both are able to contribute to increasing the digestion rate of proteins taken in with the diet. This should lead to fewer problems of dyspepsia and gastroesophageal reflux. Bromelain is a mixture of proteolytic enzymes contained in the fruit and the stem of pineapples (*Ananas comosus L. Merr.*). Bromelain also contains peroxidases, acid phosphatases, protease inhibitors. Its uses include the use in food supplements as a digestive aid thanks to its proteolytic action. Different titres are used to indicate the activity of bromelain, the most used are the following:

- GDU: Gelatin Dissolving Units
- MCU: Milk Clotting Units

One gram of bromelain standardized to 2000 MCU would correspond to 1200 GDU, the daily dose is between 200-2000 mg.

The dose of bromelain is often divided into 4 times/day.

The present composition may be used for the treatment of gastric and esophageal diseases, in particular heartburn and gastroesophageal reflux disease. The effectiveness of the composition is derived from the following component activities:

- Bicarbonates are capable of neutralizing gastric acidity by reacting with hydrochloric acid. In this way, they protect the mucous membranes from any acid-induced damage.
- The extract from the plant of the genus *Musa*, thanks to its content of chemical compounds such as pectin, phosphatidylcholine and leucocyanidin, has mucoprotettive and antiulcerogenic activities.

- The digestive enzymes, by degrading the proteins, lead to an increased stomach emptying rate. In this way, they reduce the contact of the acid with the gastric mucosa and the frequency of gastroesophageal reflux episodes, thereby preventing any injury that may result from the contact of the acid with the gastric and esophageal mucosa.

As previously stated, in the present invention the synergistic action takes place between the antacid, preferably carbonate and/or bicarbonate, the extract from a plant of the genus *Musa*, and at least one digestive enzyme, preferably actinidain, papain and/or bromelain.

In a preferred embodiment, the antacid is administered in an amount comprised between 10 mg and 500 mg, for example by administration of a dosage form in which the antacid is present at a concentration comprised between 5% and 90% w/w, more preferably in an amount comprised between 10% and 80% by weight based on the total weight of the composition; the extract from the plant of the genus *Musa* is administered in an amount comprised between 20 mg and 4000 mg, for example by administration of a dosage form in which the extract is present at a concentration comprised between 2% and 90% w/w, more preferably in an amount comprised between 5% and 85% by weight based on the total weight of the composition; the at least one digestive enzyme, which is preferably selected from actinidain, papain and/or bromelain, is administered in an amount comprised between 0.1 mg and 300 mg, for example by administration of a dosage form in which the enzyme is present at a concentration by weight ranging between 0.1% and 40% w/w, more preferably in an amount comprised between 0.25% and 30% by weight based on the total weight of the composition.

The dosage form may be a pharmaceutical composition or a supplement including the above-mentioned active ingredients mixed together, or may be a kit-of-parts for the simultaneous or sequential administration of the above-mentioned active ingredients.

The following examples are provided for illustration purposes only and do not limit the scope of the invention as defined in the appended claims.

EXAMPLES

The following examples specifically relate to the formulation of the composition of the present invention in the form of a sachet (powders or granules), however it should be understood that any pharmaceutically acceptable, preferably oral dosage form is suitable within the scope of the present invention. A preferred embodiment consists of a kit-of-parts, in which the active principles are formulated into two separate sachets, so as to ensure greater flexibility in the administration of the formulation itself. In particular, with this embodiment the digestive enzymes are administered immediately after meals, while the antacids are administered on an empty stomach, when its pH is lowered. This mode of administration maximizes the effectiveness of the combination of active principles according to the invention against gastric and gastroesophageal diseases. The extract from the fruit of the plant of the genus *Musa* in this embodiment is administered together with the antacids, however administration with the at least one digestive enzyme is also contemplated.

Some formulation examples are reported, with the quantities of the related active substances occurring in each dosage unit.

Example 1

Active ingredient	**Quantity**
Calcium carbonate	267 mg
Sodium bicarbonate	150 mg
Bromelain from Ananas comosus L., 2500 GDU/g	75 mg
Actinidain 7500 AU/g	53.32 mg
Papain 2000 USP/mg	50 mg
Musa x sapientum, d.e.	50 mg
Total (with excipients)	900 mg

Example 2

Active ingredient	**Quantity**
Calcium carbonate	267 mg
Sodium bicarbonate	150 mg
Actinidain	53.32 mg

Musa x sapientum, d.e.	50 mg
Total (with excipients)	700 mg

Example 3

Active ingredient	**Quantity**
Calcium carbonate	267 mg
Actinidain	53.32 mg
Musa x sapientum, d.e.	50 mg
Total (with excipients)	500 mg

Example 4

Active ingredient	**Quantity**
Musa x sapientum, d.e.	1750 mg
Calcium carbonate	267 mg
Actinidain	53.32 mg
Total (with excipients)	2900 mg

Example 5

Active ingredient	**Quantity**
Musa x sapientum, d.e.	1750 mg
Calcium carbonate	267 mg
Sodium bicarbonate	150 mg
Actinidain	100 mg
Total (with excipients)	2700 mg

Example 6

Active ingredient	**Quantity**
Musa x sapientum, d.e.	500 mg
Calcium carbonate	267 mg
Sodium bicarbonate	150 mg
Actinidain	20 mg

Total (with excipients)	1300 mg

Example 7

Sachet	**Active ingredient**	**Quantity**
A	Calcium carbonate	267 mg
	Sodium bicarbonate	150 mg
	Musa x sapientum, d.e.	50 mg
	Total (with excipients)	650 mg
B	Bromelain from Ananas comosus L., 2500 GDU/g	75 mg
	Actinidain 7500 AU/g	53.32 mg
	Papain 2000 USP/mg	50 mg
	Total (with excipients)	250 mg

Experimental Section

The effect of the present invention was assessed on:

- gastric emptying,
- reflux esophagitis,
- gastric secretion (volume of gastric contents, pH and total acidity),
- gastric ulcer.

Materials and Methods

Animals

Male ICR-strain mice weighing 20-25 g supplied by the company Charles River were used. The animals, housed in temperature-controlled rooms (temperature of 23±2°C, humidity 50±2%, 12-hour light-dark cycles), had free access to water and food, which consisted of a standard diet supplied by the company Mucedola Mangimi (Settimo Milanese, Italy). All experiments were performed in observance of Legislative Decree no. 116 of 27 January 1992 and according to the guidelines of the Council of the European Union (86/609/EEC and 2010/63/EU).

Gastric emptying

Gastric emptying was evaluated with the method described by Smits and Lefebvre (1996) [4]. For determining the gastric emptying, the animals were orally administered a marker (0.2 ml/mouse of a suspension containing 50 mg of phenol red in 100 ml of 1.5% carboxymethylcellulose). Twenty minutes later, the animals were sacrificed in a CO_2 saturated atmosphere and the stomach was removed. The stomach was positioned inside a test tube containing 4 ml of normal saline; after 20 seconds of stirring, 2 ml of 1 M NaOH were added to each test tube in order to obtain the maximum colour intensity. Spectrophotometric analysis (560 nm) was carried out on 1 ml of this solution. The percentage of gastric emptying was calculated according to the following formula:

100 x (1-[amount of phenol red present in the stomach after 20 min)/(amount of phenol red present in the stomach at time 0]).

Reflux esophagitis and gastric secretion/ulcer

Reflux esophagitis and gastric secretion/ulcer were induced by using the experimental pylorus ligation model described by Shay et al. (1945) [5]. The animals, fasted for 24 hours but with free access to water, were anaesthetised, the abdomen was opened and the pylorus

was ligated. Four hours after the surgical procedure (the time required to cause submaximal injury of the esophageal mucosa) [6], the mice were sacrificed in a CO_2 saturated atmosphere and the esophagus and stomach were removed for assessing: 1) macroscopic esophageal and gastric damage, 2) degree of esophageal and gastric inflammation (myeloperoxidase activity), and 3) characteristic parameters of gastric secretion (volume of the gastric contents, pH and total acidity).

1) Macroscopic esophageal and gastric damage

The esophagus (opened longitudinally) and stomach (opened along the greater curvature) were spread out on a polystyrene support and analysed with the aid of a microscope for the detection of mucosal injury. Mucosal damage was determined by using a scoring scale that takes into account the severity and extent of hyperaemia and haemorrhagic erosions.

2) Degree of inflammation of the esophagus and stomach

The degree of inflammation of the esophagus and stomach was assessed by determining myeloperoxidase activity (MPO). MPO is a protein found in the azurophilic granules of polymorphonuclear neutrophils and is used as a marker of leukocyte infiltration. In order to assess MPO activity, the tissues were subjected to three homogenisation cycles of 10 seconds each, at maximum speed, in a suitable lysis buffer designated as MOPS (0.5% HTAB in 10 mM MOPS), in a ratio of 50 mg tissue/1 ml MOPS. The homogenates underwent centrifugation at 12,000 rpm for 20 min at 4°C. Subsequently, the supernatants were incubated with NaPP (sodium phosphate buffer pH 5.5) and 16 mM TMB (tetramethylbenzidine) followed, after a five-minute incubation at room temperature, by addition of H_2O_2 diluted in NaPP. The reaction was quenched with 2M cold acetic acid and 1 ml of the reaction solution was spectrophotometrically read at a wavelength (λ) of 650-655 nm. The values obtained were compared to a standard MPO curve and the results expressed as U/ml of MPO.

3) Assessment of the characteristic parameters of gastric secretion

The gastric contents were collected for determining the pH value and the total amount of gastric juice (volume). Subsequently, 2 ml of distilled water were added to the gastric contents, which were centrifuged at 5000 rpm for 15 min in order to determine the total acidity, which was carried out on the supernatant by titrating to pH = 7 (using 2% phenolphthalein as an indicator) with 0.01 N NaOH. The total acidity was expressed as mequiv.[H+]/ml/4 h.

References

[1] El-Serag HB, Sweet S, Winchester CC, et al. Update on the epidemiology of gastro-oesophageal reflux disease: a systematic review. Gut 2014; 63: 871–80.

[2] Harnik Ian G. Gastroesophageal Reflux Disease Ann Intern Med. 2015;163(1):ITC1.

[3] Boeckxstaens G, El-Serag HB, Smout AJ, Kahrilas PJ. Symptomatic reflux disease: the present, the past and the future. Gut. 2014; 63(7):1185-93.

[4] Smits GJ, Lefebvre RA. Influence of aging on gastric emptying of liquids, small intestine transit, and fecal output in rats. Exp Gerontol. 1996; 31(5):589-96.

[5] Shay H, Komarow SA, Fels SS, Meranze D, Gruenstein M, Siplet H. A simple method for the uniform production of gastric ulceration in the rat. Gastroenterol 1945; 5:43.61

[6] Takeuchi K, Nagahama K. Animal model of acid-reflux esophagitis: pathogenic roles of acid/pepsin, prostaglandins, and amino acids. Biomed Res Int. 2014; 2014:532594.

CLAIMS

1. A composition comprising the combination of at least one antacid, an extract from the fruit of a plant of the genus *Musa*, and at least one protease digestive enzyme.

2. The composition according to claim 1, wherein the at least one antacid is selected from the group consisting of carbonates, bicarbonates, citrates, hydroxides, metal oxides, trisilicates and any combination thereof, and the antacid is preferably present in the composition in an amount comprised between 5% and 90% by weight based on the total weight of the composition, more preferably in an amount comprised between 10% and 80% by weight based on the total weight of the composition.

3. The composition according to claim 1 or 2, wherein the plant of the genus *Musa* is *Musa paradisiaca* or *Musa sapientum*, and the extract is preferably present in the composition in an amount comprised between 2% and 90% by weight based on the total weight of the composition, more preferably in an amount comprised between 5% and 85% by weight based on the total weight of the composition.

4. The composition according to any of claims 1 to 3, wherein the at least one digestive enzyme is selected from the group consisting of papain, actinidain and bromelain, and the digestive enzyme is preferably present in the composition in an amount comprised between 0.1% and 40% by weight based on the total weight of the composition, more preferably in an amount comprised between 0.25% and 30% by weight based on the total weight of the composition.

5. The composition according to any of claims 1 to 4, which is formulated as an oral pharmaceutical formulation.

6. The composition according to any of claims 1 to 5, for use in the prevention and/or treatment of gastric or gastroesophageal diseases.

7. The composition for use according to claim 6, wherein the gastric or

gastroesophageal diseases are selected from dyspepsia, heartburn and gastroesophageal reflux disease.

8. A kit-of-parts for the sequential or simultaneous administration of at least one antacid, an extract from the fruit of a plant of the genus *Musa*, and at least one protease digestive enzyme, for use in the prevention and/or treatment of gastric or gastroesophageal diseases preferably selected from dyspepsia, heartburn and gastroesophageal reflux disease.

9. The kit-of-parts according to claim 8, wherein the at least one antacid is as defined in claim 2, the extract from the fruit of a plant of the genus *Musa* is as defined in claim 3, and the at least one protease digestive enzyme is as defined in claim 4.

10. The kit-of-parts according to claim 8 or 9, which is formulated as an oral pharmaceutical formulation.

ABSTRACT

A composition for use in the prevention and/or treatment of gastric or gastroesophageal diseases

The invention relates to a synergistic composition of naturally occurring substances, which is particularly effective in the treatment and prevention of gastric or gastroesophageal diseases. The composition of the invention comprises the synergistic combination of at least one antacid, an extract from a fruit of a plant of the genus *Musa*, and at least one protease digestive enzyme. The synergistic composition of the present invention may be provided in the form of a pharmaceutical composition or a food supplement, or in the form of a kit-of-parts.