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Chapter

# Role of Alginates Combined with Natural Extracts to Prevent the Gastric Acid-Related Damage

*Francesca Uberti, Lorenzo Secondini, Ian Stoppa, Mietta Catera and Claudio Molinari*

## Abstract

The human stomach is extremely vulnerable to various attacks able to cause erosion and mucosal epithelium damage which lead to gastrointestinal tract bleeding and/or ulcer perforations and finally worsen the original disease. A prolonged exposition to strong acidic environment causes coagulation necrosis resulting from the desiccating action of the acid on proteins in exposed tissues with inflammation and accumulation of intracellular radical oxygen species. Therapeutic strategies aim to treat both symptoms and epithelial damage with chemical or mechanical approaches. In this context, alginates seem to have great importance, especially if combined with other molecules known to have some properties on gastric epithelial cells, for example, vitamin D3, extract of prickly pear and olive leaves, and a tyndalized probiotic. This natural composition is able to exert a gastroprotective effect to maintain or restore the integrity of gastric epithelium through an antioxidant pathway, inhibiting apoptosis and activating survival kinases better than other pharmacological or natural active principles.

**Keywords:** alginates, vitamin D3, prickly pear, olive leaves, tyndalized probiotic

## 1. Introduction

The impact of gastric diseases on human health is a worldwide problem in modern society [1, 2]. For example, in the USA, studies have reported gastrointestinal illness rates in the range of 0.5–2 episodes/year/person and incidence of 5–100 episodes/1000/week according to seasons and age. The number of episodes of gastrointestinal illnesses is similar in both 40-year-old studies and in recent ones [3, 4]. The gastrointestinal disorder, including chronic gastritis, duodenal and gastric ulceration, adenocarcinoma, and gastric mucosa-associated lymphoid tissue (MALT), may have a common cause which is gastroesophageal reflux disease (GERD). The cardinal symptom is heartburn caused by the involuntary movement of gastric contents to the esophagus that occurs several times a day, mainly in postprandial period [5]. In particular, most reflux episodes are of short duration, asymptomatic, and limited to the distal esophagus [6]. However, some episodes occur with typical symptoms such as burning sensation in the chest that can also extend to the throat, gastric pain, episodes of regurgitation, dysphagia, and sensation of bolus in the throat [7]. One of the risk factors is bad eating habits that



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can aggravate gastroesophageal reflux and can contribute to the delayed gastric emptying with an increase of acid secretion in the stomach [8]. In the human stomach, the acid environment (pH 1 to 2) acts both as a primary defense against infections and intervenes in the early stages of digestion [9]. The gastrointestinal epithelium is a fundamental barrier protecting the gastrointestinal mucosa from damage through the ability of epithelial cells to spread and migrate across the basement membrane to repair the damage [4]. However, gastric acid (HCl) secreted from gastric parietal cells has been reported to determine gastric mucosal injuries, and, consequently, a prolonged exposition to strong acidic environment causes coagulation necrosis in exposed tissues [10]. A mild gastritis condition can cause long-lasting damage resulting in cellular injury which in turn causes inflammation [11] that creates further free radical-dependent tissue destruction [12, 13]. This injury involves DNA and can lead to stomach cancer genesis [14]. Some recent studies have shown that non-steroidal anti-inflammatory drugs (NSAIDs) are one of the most common causes of damage on the gastric mucosa through the production of free oxygen radicals (ROS) leading to lipid peroxidation and apoptosis [10, 15] of cells. In particular, superoxide anion ($O^{-2}$) produced by mitochondria is the main cause of oxidative damage [16] and plays a critical role in the pathogenesis of gastric disorders [17]. Around 30 million people consume NSAIDs globally every day [18] since they are prescribed because of their efficacy in the management of pain, inflammation, and fever [19]. Adverse effects associated with NSAIDs, such as alterations in renal function, effects on blood pressure, hepatic injury, and platelet inhibition, are a challenge in clinical treatment optimization [20]. However, gastric mucosal perforation and bleeding are a major concern as well as the worst outcome of prolonged NSAID therapy [21]. NSAIDs are able to induce gastric mucosal lesions because of their acidic properties [22]. The mechanism behind gastric damage involves a highly acidic gastric environment that favors the migration of nonionized lipophilic NSAIDs into the epithelial cells [23]. Diclofenac is the most widely prescribed NSAID for treating pain, inflammation, and several forms of cancer [24, 25]. The main clinical problem exerted by diclofenac is toxicity related to oxidative injury of tissues, which appears to play a role in the pathophysiology of digestive tract ulceration [18, 25, 26]. For this reason, in order to prevent the damage, NSAID treatments are usually associated to proton-pump inhibitors (PPI) [27]. However, the anatomical and functional integrity of gastric mucosa rely on the balance between aggressive and defensive mechanisms [19]; for this reason, the success of pharmacological treatments in prevention or healing of gastric lesions depends both on the blockade of acid secretion and on the enhancement of mucosal protective factors [19]. In addition, current evidence suggests that PPI are also associated with numerous side effects such as hypergastrinemia, enteric infections, adverse cardiovascular events, and increased mortality rates [23]. Basing on these data, it can therefore be assumed that gastric acid can induce ROS production, lipid peroxidation, and apoptosis through a mechanism similar to that observed by NSAIDs [10]. For this reason, ROS, including $H_2O_2$, are a major cause of cellular oxidative damage [16], and they play a critical role in the pathogenesis of gastric disorders [2, 17]. After a gastric mucosa damage, several events occur in order to restore the integrity of the layer; normal epithelial repair consists of restitution of functions and regeneration of anatomical integrity involving epithelial proliferation followed by a remodeling phase [28]. During restitution, epithelial cells spread through the basement membrane to restore cell continuity, a process that is independent of cell proliferation. The clinical need to prevent or restore the damage of gastric mucosa in early stage encourages the search of novel agents able to counteract both the adverse effects of drugs and hyperacidic conditions with a better safety profile, in particular using natural compounds. A new group of widely used molecules

includes alginates, a polymer of alginic acid derived from the cell wall of various brown algae. Alginate-based compounds have been available for several decades [29] and are listed as inactive ingredients with a substantial and independent benefit from alginate formulation [29]. Recently, there is a new growing interest in alginates as a therapy for GERD, including patients with continued symptoms despite acid suppression therapy [30]. Alginates are polysaccharides composed of two β-d-mannuronic acid (M) and α-1-glucuronic acid (G) monomers which are held together by β 1,4 bonds; these monomers are not organized in repeated units, and they can be considered as a set of heterogeneous molecules since they are distributed as several different repetitions called M, G, or MG blocks [31, 32]. These alginates are able to block the HCl reflux in a mechanic manner; indeed, they do not have any pharmacologic property [33]. The mechanism of action of alginates has been called "rafting", which means that in the presence of gastric acid, they form a gel in which carbon dioxide (resulting from the splitting of bicarbonate) is trapped. Then the gel is carried to the top of the gastric contents neutralizing the acidity and preventing the ascent of acid material into the esophagus [34]. The advantage of alginate-based reflux suppressants over antacid alone is that they provide rapid and longer-lasting symptom relief [35].

Subsequently, it was seen that it is useful to associate alginates with natural substances. This association has been found to be effective in promoting stomach health, reducing inflammation, and supporting the immune system. Zinc, a micronutrient involved in multiple functions, is able to act as a coenzyme on several enzymes that protect cells against free radical damage [36]. Furthermore, zinc has direct effects against inflammation, as it helps to stabilize the membrane of mast cells, responsible for inflammation [37]. Other natural products with antioxidant property seem to play an active role in the stomach well-being, such as blueberries or licorice, that they are able to reduce the production of cytokines and at the same time to increase the production of the mucous membrane of the stomach [38]. Moreover alginate/antacid system has been used as a carrier of probiotic, drugs, and plant extracts [39]. For example, alginate/bicarbonate combined with two herbal gastroprotective extracts (*Opuntia ficus-indica* and *Olea europaea*) has been successfully evaluated in patients with GERD [39]. However, the most common treatments for gastroesophageal reflux includes different molecules such as magnesium hydroxide or aluminum hydroxide (commercially named Maalox®) in which they absorb gastric acidity, reducing the pH and reducing/reversibly blocking acid secretion by parietal cells. The effectiveness of these preparations is due to their ability to exert a buffering effect on the gastric pH [40], but the adverse effect is that they can cause an accumulation of aluminum which is a common cause of neurodegeneration and neurotoxicity [40].

Recent studies have explored a possible role of vitamin $D_3$, the active form of vitamin D, on gastroprotection as one of the extra skeletal effects this vitamin has [4, 23]. Vitamin $D_3$ binds its receptor VDR which is present in several tissue targets in the digestive system, in particular in the oral region, and in epithelial cells of the oral cavity, tongue, and gums. In addition, vitamin $D_3$ appears to have a therapeutic role in gastric mucosa as well, stimulating cell proliferation and differentiation [41] and regulating endocrine/paracrine gastrin and pepsinogen secretions [42]. Finally, vitamin $D_3$ acts on smooth muscle cells in the pyloric region and in different areas of the small intestine [42]. Furthermore, the low plasmatic level of vitamin $D_3$ is found to be responsible for an insufficient emptying of the stomach, swelling, constipation, and intestinal irritation [42]. After the binding between vitamin $D_3$ and its receptor, several intracellular events involved in different mechanism start, including the protective role against oxidative stress [43], the regulation of autophagic pathways through the regulation of ATG16L1, a protein complex necessary for autophagy [44],

and the ability to inhibit apoptosis by increasing the expression of endothelial nitric oxide synthase (eNOS) leading to the nitric oxide (NO) production [43]. In this context, vitamin $D_3$ can exert some beneficial effects on gastric tissue. For this reason, its use in association with other gastroprotective agents such as alginates can increase therapeutic efficacy of the formulation in respect to the efficacy that would be obtained only with gastroprotective drugs. Indeed, this effect is described in a recent study in which this association (vitamin $D_3$ and raft-forming alginates, buffers, polysaccharides, and biophenols named Aquilea Reflux®) could be of greater efficacy when compared to other gastroprotectants (e.g., Maalox® or Gaviscon®) or other natural extracts (e.g., Neobianacid®) [4, 23]. Aquilea Reflux® is a dietary supplement that combines the properties of calcium alginate in a buffer solution, resulting from alkaline salts useful to counteract situations of high acidity, with a tyndalized probiotic (Pylopass®) and an extract of prickly pear and olive leaves (Mucosave®). The extract of prickly pear is useful for its emollient and soothing characteristics at the level of the digestive system. All these agents were added to vitamin $D_3$. This study demonstrates for the first time that the combination between alginate-based gastroprotector agent and vitamin $D_3$ has a beneficial effect joining the effects of a mechanical barrier with the modulation of intracellular pathways in order to maintain or restore the integrity of gastric epithelium. Indeed, Aquilea Reflux® is able to improve the adhesivity of cells which is crucial on cell migration involving two important extracellular matrix glycoproteins, vitronectin and fibronectin. This is more important because it indicates that vitamin $D_3$ is able to improve the beneficial effects induced by alginates, thus supporting data about the mechanism of gastroprotection induced by alginates [45]. Another important element is a time of adhesion, which is an important reparative event; the combination is able to occur rapidly, confirming other data about the reparative events following acute gastric injury [46]. Since there is evidence that oxidative stress plays an important role in the pathogenesis of acute gastric injury [47–49], Aquilea Reflux® has been also tested to verify if it is able to improve both cell viability and cell proliferation after $H_2O_2$ or HCl exposure. This combination significantly reduced ROS production and decreased cell viability loss, suggesting that cell damage and cytotoxicity can be reduced.

These results suggest that it may exert a better gastroprotective effect through an antioxidant pathway, inhibiting apoptosis and activating survival kinases. Such effect was stronger in preventing epithelial damage than what was observed using other gastroprotective agents such as Gaviscon®, Maalox®, or proton-pump inhibitors. Ultimately, it can be said that alginate-based gastroprotectors combined with vitamin $D_3$ have beneficial properties on gastric epithelial cells, joining the effects of a mechanical barrier with the modulation of intracellular pathways in order to maintain or restore the integrity of gastric epithelium. In addition, comparing the activity to other natural products, such as Neobianacid® which is a mixture of polysaccharides and flavonoids able to improve the protection of the stomach and the esophagus thanks to the presence of Poliprotect® and a flavonoid fraction (*Matricaria recutita* and *Glycyrrhiza glabra*), the combination composed of alginates plus vitamin D3 appears to have significant effects. Indeed, this combination significantly reduced ROS production and decreased cell viability loss after the injury, suggesting that cell damage and cytotoxicity can be prevented, exerting a better gastroprotective effect through an antioxidant pathway, inhibiting apoptosis, and activating survival kinases. Such effect was stronger in preventing epithelial damage than what was observed using other gastroprotective agents such as Neobianacid® (**Figure 1**).

These data show that it is possible to improve the beneficial effects of alginates by combining active ingredients that are capable of intervening at the cellular

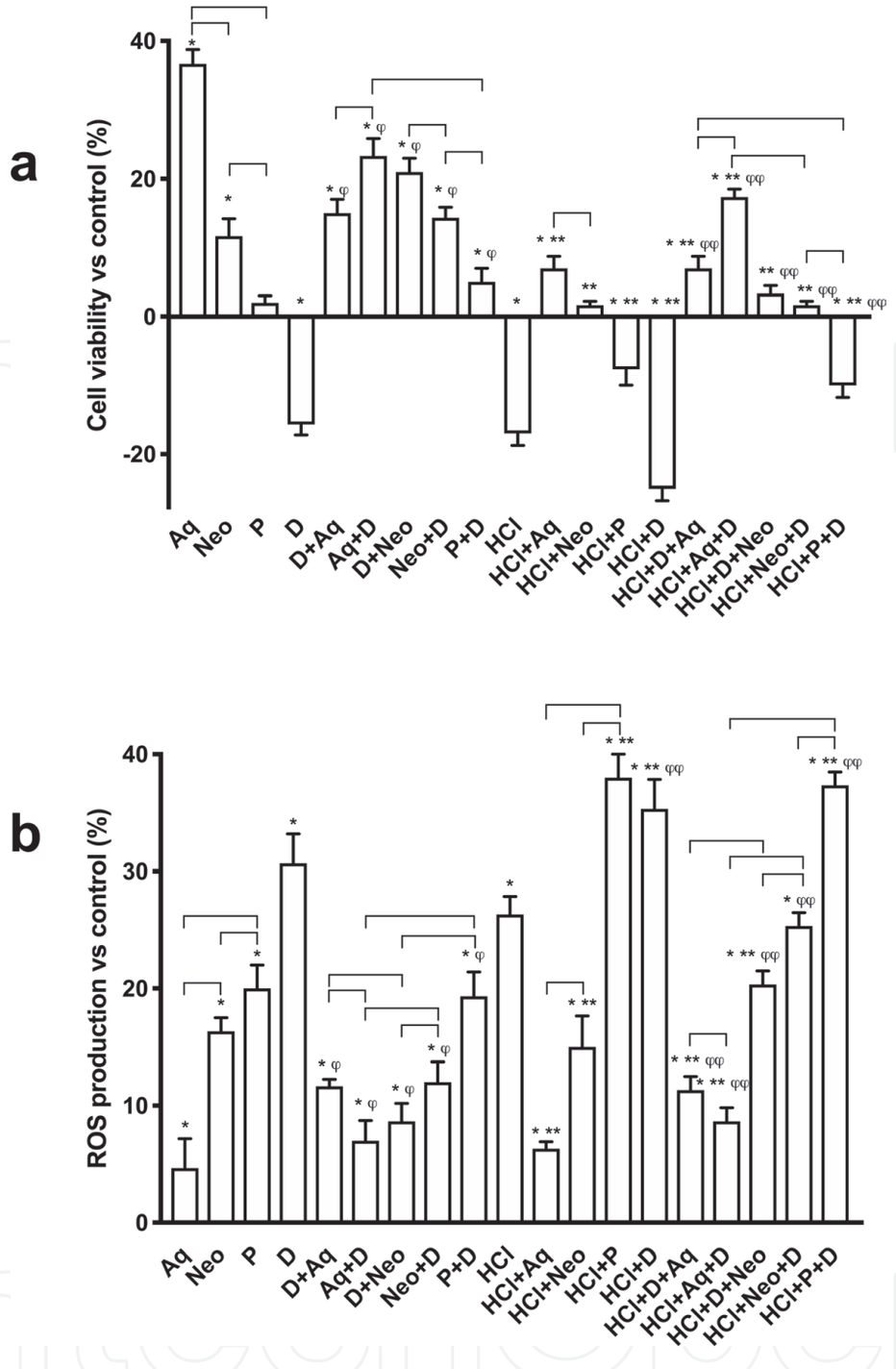



**Figure 1.**
*Effects of Aq and Neo alone and combined with diclofenac during acidic and hyperacidic conditions. In panel A cell viability and in panel B ROS production observed in gastric epithelial cells treated for 24 h. P= pantoprazole, D= diclofenac. The other abbreviations are similarly reported in the figure. Data are expressed as means ± SD (%) of five independent experiments normalized to control values. *p < 0.05 vs. control; **p < 0.05 vs. HCl; φp < 0.05 vs. diclofenac; φφp < 0.05 vs. HCl + diclofenac; arrows indicate p < 0.05 between different groups.*

level to improve viability and to reduce the production of ROS, from which significant damage to the gastric mucosa originates. The formulation called Aquilea Reflux®, with its combination of chemical, mechanical, and biological agents, has proven to be effective in preventing cellular alterations caused by NSAIDs in both acid and hyperacidic conditions, reducing ROS production and apoptotic mechanism, and increasing the activation of survival kinases and cell proliferation.

## 2. Conclusions

In conclusion it can be stated that the antacid and gastroesophageal reflux abilities of alginate-based preparations could be significantly improved by combining natural extraction components and vitamin D3, providing an association of chemical, mechanical, and cellular action to achieve a complete protective effect.

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## Conflict of interest

Lorenzo Secondini and Mietta Catera are employees of Laborest Spa; however, they provide an unbiased contribution to this study.

## Acronyms and abbreviations

| | |
|---|---|
| HCl | gastric acid |
| GERD | gastroesophageal reflux disease |
| NSAID | non-steroidal anti-inflammatory drugs |
| PPI | proton-pump inhibitors |
| ROS | free oxygen radicals |
| eNOS | endothelial nitric oxide synthase |
| NO | nitric oxide |

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