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Evaluating the Protective Effects of a Pet Supplement Containing Calcium Carbonate, Magnesium Hydroxide and Natural Ingredients (*Gastrik Pet*®, Candioli S.r.l.) on Gastric Cells and Cytotoxicity Reduction under Hyperacidity Conditions: An In Vitro Study

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Abstract:

Effective acid suppression is crucial for treating gastritis in pets. This in vitro study aimed to evaluate and compare the protective effects of two products on gastric cells under conditions of high acidity: 1) a supplement (*Gastrik Pet*®, Candioli srl) containing selected natural ingredients including Promugel®(a complex of mucilage protecting the mucosa from gastric acidity: Guar meal, Psyllium (*Plantago Ovata* L.), *Trigonella foenum-graecum L.*) and antacid salts (*calcium carbonate* and *magnesium hydroxide*) and 2) sucralfate, a treatment used in human and veterinary medicine. *Gastrik Pet*® showed superior efficacy in protecting gastric cells from acid-induced cytotoxicity at all concentrations tested compared to sucralfate. These results suggest that this natural pet supplement could be a promising option for managing gastritis-related conditions in pets. Further research, including in vivo studies, is needed to confirm these findings and assess its clinical potential.

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1. INTRODUCTION

Gastritis is a gastric condition marked by acute or chronic vomiting, resulting from inflammation of the gastric mucosa [1-3]. Effective management of gastritis depends largely on acid suppression to prevent mucosal damage from the acidic environment and reduce the risk of gastric ulcers [4,5].

In cases of gastric inflammation, factors such as chemical injury, ischemia, infection, or exposure to antigens can activate the release of inflammatory mediators and vasoactive substances. This process disrupts gastric epithelial cells, increases acid secretion, and weakens the gastric barrier [2,6]. The resulting inflammatory cascade further promotes acid secretion, damages the mucosa, raises cell membrane permeability, and affects microvascular blood flow [6]. Normal gastric secretions, which include acid, mucus, bicarbonate, and antibacterial agents, form the body's primary defense against gastric irritants. The gastric epithelium also plays a protective role by serving as a barrier to acid diffusion and facilitating cell repair following injury [6]. Gastric juices are essential to the digestive process, with their acidity being a natural component of digestion's physiology [7]. However, hyperacidity can occur due to factors such as dietary choices, infections, or lifestyle habits. At the cellular level, excessive gastric acid can damage the stomach lining, resulting in inflammation and clinical symptoms such as gastroesophageal reflux and dyspepsia.

Gastroprotective products are essential in neutralizing excessive acidity, restoring the gastric environment to normal physiological levels, and supporting overall gastric function. These products help protect the gastric mucosa from cytotoxic damage caused by hyperacidity.

In veterinary medicine, several therapeutic approaches are used to lower gastric acidity and enhance mucosal protection, with the goal of preventing mucosal injury. These options include Histamine-2 (H2) receptor antagonists, proton pump inhibitors (Tolbert, Davis) [4,5], as well as misoprostol, sucralfate, and insoluble salts [8].

Sucralfate is a complex salt that has demonstrated effectiveness in aiding gastric mucosal tissue repair in dogs following acid-induced injury [8, 9]. Its action in managing acid-peptic disease is multifaceted. It not only forms a protective barrier but also stimulates prostaglandin production in the gastric epithelium, making it useful for treating conditions like erosive esophagitis [8]. Sucralfate is generally considered safe, with minimal side effects, such as constipation and, less frequently, nausea and vomiting [8, 10]. Studies also highlight the potent effects of natural products in combination with antacid salts, which offer potential anti-inflammatory, antioxidant, and cytoprotective benefits [11,12].

This *in vitro* study aims to compare the efficacy of a pet supplement (*Gastrik Pet*®, Candioli srl) containing selected natural ingredients including Promugel®(a complex of mucilages protecting the mucosa from gastric acidity: Guar meal, Psyllium (*Plantago Ovata* L.), *Trigonella foenum-graecum L.*) and antacid salts (*calcium carbonate* and *magnesium hydroxide*) with that of sucralfate. Sucralfate is a medication commonly used in both veterinary and human medicine. Specifically, we assessed the ability of these two products to protect gastric cells and reduce cytotoxicity in conditions of hyperacidity.

2. MATERIAL & METHODS

2.1. Cellular Lines and Culture Conditions

The experiments were conducted on a cell line of immortalized human gastric epithelial cells (AGS cells). The cells were cultured at 37 °C, 5% CO_2 in MEM/F12 medium, supplemented with 1% L-glutamine and 10% FBS (Fetal Bovine Serum).

2.2. Products and preparation of Treatments

Gastrik pet® tablets (Candioli Pharma srl) were crushed using a mortar to obtain a homogeneous powder.Then, a 10x stock solution (54 mg/ml) was prepared resuspending the powder in PBS (Phosphate Buffered Saline) buffer and subsequent dissolution for 1 hour at 37°C. The range of concentrations used for the assays was established on the basis of the therapeutic indications for the destination of use (one 2 grams' tablet for cats or dogs).

Sucralfate granulate (DOC Generici) was prepared in PBS according to the therapeutic indications, obtaining a 10x solution (54 mg/ml).

The list of ingredients of *Gastrik pet*® tablets (Candioli Pharma srl) is reported in Table **1**.

2.3. Cellular Viability Assay

AGS cells were seeded into 96-well plates at a density of 10^4 cells/well in growth medium cells per well

Table 1: Ingredients of the Two Products Tested in the Study *Gastrik pet*® (Candioli Pharma srl) and Sucralfate

PRODUCT for veterinary medicine (Gastrik pet®, Candioli Pharma srl)			
Ingredients		**%**	**mg/2g tablet**
Microcrystalline cellulose		38.5	770
Yeasts, inactivated		13.4	268
Palatability enhancer (Optimizor Uranus)	Sodium pyrophosphate	5.7	114
	Yeasts, inactivated	3	60
	Lupin protein meal	1.21	24.2
	Sunflower oil	0.09	1.8
L-threonine		6.3	126
Calcium carbonate		5	100
Magnesium hydroxide		3.8	76
Methyl sulphonyl methane		3.1	62
Thea sinensis L. = Camellia thea Link. = Camellia sinensis (L.) O. Kuntze: Tea extract		3	60
Mono and diglycerides of fatty acids (Glyceryl dibehenate)		2	40
Colloidal silica		1.5	30
Magnesium stearate		1.5	30
Products from the processing of plant (Aloe vera)		0.6	12
Glycyrrhiza glabra L.: Licorice extract (wb)		0.6	12
Promugel®: Guar meal, Psyllium – Plantago Ovata L. powder cuticle, Trigonella foenum-graecum L. fenugreek extract		10.7	214
Total weight of a tablet (2g)			
Product for human medicine (Sucralfate)			
Sucralfate		100%	2

supplemented with 10% FBS. After 24 hours, the medium was replaced with 100 µL of complete medium containing 1% FBS for treatment with sucralfate. For the treatment, cells were exposed to 10 different concentrations, prepared through a series of 1:2 serial dilutions, to determine non-toxic doses for the bioactivity test. Sodium dodecyl sulfate (SDS) at 1 mg/mL served as a positive control. The cells were incubated for 24 hours at 37°C with 5% CO_2. The following day, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was added to each well at a final concentration of 0.5 mg/mL, and the plate was incubated for an additional 2 hours under the same conditions. Afterward, reduced MTT crystals were dissolved by removing the medium and adding 100 µL of DMSO to each well. The absorbance at 595 nm was measured using an Infinite M NANO+ plate reader (Tecan), with cellular viability expressed as a percentage of the absorbance at 595 nm of untreated (NT) cells. This assay was also detailed by Lonigro and colleagues (2024) [12], where it was used to evaluate the tested products.

2.4. Acidic pH Gastroprotection Test

The assay was performed as described in Lonigro *et* al, 2024. Briefly, AGS cells were seeded into 96-well plates at a density of 10^4 cells/well in growth medium containing 10% FBS. After 24 hours, the medium was replaced with complete medium containing 1% FBS. The cells were then pre-treated with either *Gastrik pet*® or Sucralfate at the concentrations as per the indication, for one hour. Following this, acid conditions were simulated by exposing the AGS cells to 1M hydrogen chloride (HCl), adjusting the pH to 2. This acidic incubation was maintained for one hour, after which an MTT assay was conducted as previously described. The experimental setup included a negative control group of untreated cells (NT) and a group exposed only to hyperacidic conditions without any protective treatment. Gastroprotective effects of the tested products were evaluated by comparing cell viability between well treated solely with HCl (representing hyperacidic conditions) and those treated with the products.

2.5. Statistical Analysis

The data were statistically analyzed through the GraphPad Prism software, by one-way ANOVA, followed by Dunnett's post-test. Each experimental group was compared with the positive control (pH 2).

3. RESULTS & DISCUSSION

3.1. Cellular Viability, Cytotoxicity Test

Cell viability following treatment with Sucralfate was analyzed to determine non-toxic concentrations for AGS cells, which would be used in subsequent experiments. For the *Gastrik pet*® product, the non-toxic concentrations were identified in previous tests [12] and were used in this study.

The results show that the Sucralfate product always determines cell viability higher than 88%, for all the concentrations tested compared to the untreated control (Figure **1**). As expected, the positive toxicity control (tox) shows a significant reduction in cell viability, confirming the validity of the test (Figure **1**).

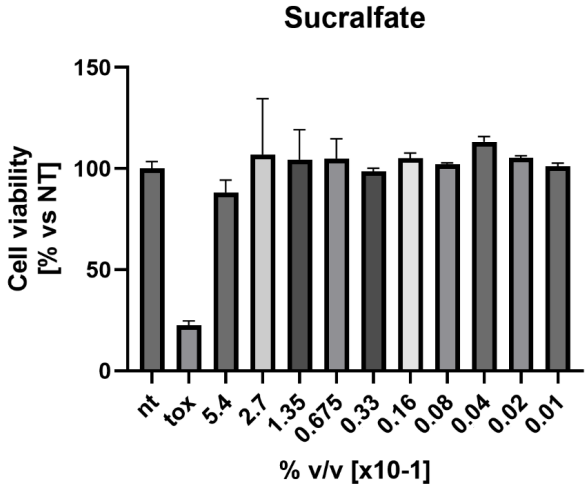

Figure 1: Cytotoxicity Test for the Sucralfate. The result is expressed as cellular viability (%) compared to the untreated control (nt).

3.2. Acidic pH Gastroprotection Test

In vitro studies assessing the gastroprotective efficacy of natural products have become essential precursors to in vivo research [13, 14]. For example, in a recent study that built on prior cytotoxicity testing, cells were treated with the three highest concentrations of various tested products [12]. Among these, *Gastrik pet*® demonstrated the strongest efficacy in protecting gastric cells from hyperacidity-induced cytotoxicity [12].

In a similar approach to the study by Lonigro and colleagues (2024) [12], a new assessment was conducted in the current research to compare the gastroprotective efficacy of *Gastrik pet*® and Sucralfate. Cells were treated with the following concentrations:

Gastrik pet®: 0,675 – 0,338 – 0,169 x10-1% v/v

Sucralfate: 0,675 – 0,338 – 0,169 x10-1% v/v

The cell treatment was carried out for 1 hour, before the induction of the gastric hyperacidity condition. Then, the culture's medium pH was acidified, and the cells were incubated for another hour.

At the same time, two additional experimental groups were included: a group of untreated cells (NT) and a group of cells only treated with hyperacidic condition. At the end of the incubation, cell viability was analyzed. As shown in Figure **2**, the hyperacidity condition causes a reduction of approximately 25% in cell viability.

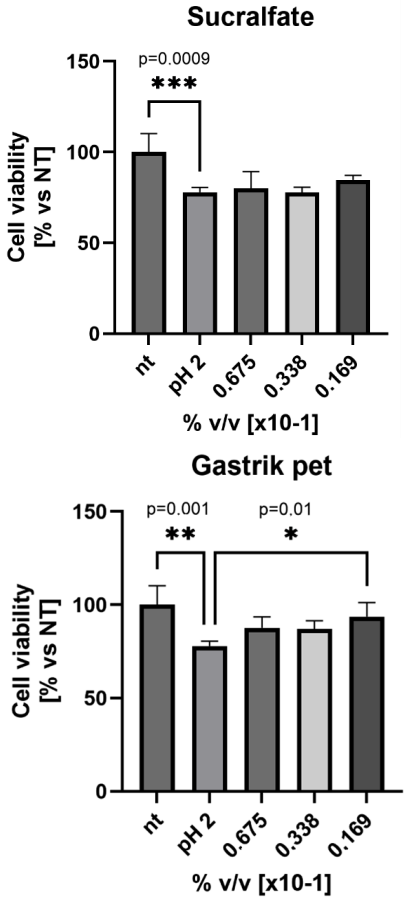

Figure 2: Acid pH gastroprotection test at different concentrations for the two products (sucralfate and *Gastrik pet*®). The result is expressed as cell viability (%) compared to the untreated control (nt). ***p<0.001 vs pH 2; **p<0,01 vs pH 2; *p<0,05 vs pH 2.

As shown in Figure **2**, the *Gastrik pet*® product demonstrated a potential cytoprotective effect across all three concentrations tested, with cell viability ranging from 87% to 94%, compared to approximately 78% viability in cells exposed to hyperacidic conditions. Notably, the 0.169% concentration produced a modest although statistically significant cytoprotective effect, with a p-value of 0.01 vs pH 2, thus below the 0,05 threshold.In contrast, for Sucralfate, cell viability remained between 80% and 85%, closer to the 78% observed in hyperacidic conditions alone. The results are summarized in Table **2**, where a direct comparison of cell viability is shown for both *Gastrik pet*® and Sucralfate under hyperacidic conditions (pH 2). Moreover, the Table **2** shows the difference of cell viability between *Gastrik pet*® or Sucralfate treated group and the positive control group (pH 2). The latter comparison highlights that *Gastrik pet*® exerted a higher cytoprotective effect at all tested concentrations.

Sucralfate represents the standard of care due to its film-forming capacity on the gastric mucosa. However, when used at the same concentration as *Gastrik pet*® *in vitro,* it didn't show a cytoprotective effect on gastric cells. The mechanism of action of sucralfate in acid-peptic disease is multifactorial. It forms stable complexes with proteins in damaged mucosa, where there is a high concentration of exudate-derived proteins such as fibrinogen, albumin, or globulins (Marks, 2018). In a study on dogs, sucralfate effectively promoted the repair of gastric mucosal tissue when applied immediately or shortly after acid-induced injury. Additionally, studies in rabbits, cats, and humans have demonstrated sucralfate's protective effects on oral, esophageal, and gastric ulcers (Marks, 2018). Given this premise, our findings underscore the potential relevance of the cytoprotective effects observed with *Gastrik Pet*® within our experimental setup.

The development of robust in vitro models for evaluating gastroprotective effects offers numerous advantages beyond reducing the reliance on laboratory animals. These models allow researchers to closely examine the cellular and molecular mechanisms of gastric protection in a controlled environment. This facilite precise assessments of how specific compounds interact with gastric tissues. Furthermore, they provide a rapid and cost-effective screening method for identifying potential gastroprotective agents early in the drug development process. This can help to filter out less effective or toxic compounds before advancing to more complex and resource-intensive in vivo studies.

Validated in vitro models can also enhance predictive accuracy for pharmacological outcomes, allowing researchers to observe dose-response relationships, cellular recovery processes, and cytoprotective efficacy with high reproducibility. This predictive power holds significant promise for advancing gastroprotection research.It enables scientists to simulate various physiological conditions, such as hyperacidity or oxidative stress, to assess how compounds might perform under these challenges. As a result, these models could become invaluable tools in streamlining drug discovery, optimizing compound selection, and accelerating the development of effective gastroprotective therapies while reducing the need for animal testing.

In agreement with our results, other two recent *in vitro* studies have explored the synergistic effects of antacid salts (*calcium carbonate* and *magnesium hydroxide*) and selected natural ingredients (*Gastrik pet*®, Candioli srl), on acid suppression [11,12]. One study demonstrated that this product exhibited stronger acid-suppressant activity and provided greater protection against gastric cell cytotoxicity than other products tested [11]. Another study confirmed once again that the presence of *calcium carbonate* and *magnesium hydroxide* together with other natural ingredients, was more effective in preventing acid-induced cytotoxicity in

Table 2: Comparison between *Gastrik pet*® and Sucralfate groups in terms of: (i) gastric cell viability in hyperacidic conditions (pH 2), and (ii) gastric cytoprotection, expressed as a difference of cell viability between the *Gastrik pet*®-treated or Sucralfate-treated groups and the positive control group (pH 2).

	Cell Viability [Mean % vs nt]		Cytoprotection [% Cell Viability Difference vs pH 2]	
Concentration	*Gastrik pet*®	Sucralfate	*Gastrik pet*®	Sucralfate
0.675%	87.66	80.01	9.69	2.04
0.338%	87.12	77.87	9.15	-0.09
0.169%	93.71	84.74	15.75	6.78

gastric cells, showing a dose-dependent trend in efficacy[12]. In particular, this pet supplement contains selected natural ingredients called Promugel®(Guar meal, Psyllium (*Plantago Ovata* L.), *Trigonella foenum-graecum L.*). The effect of *Trigonella foenum-graecum* on ethanol-induced gastric ulcer wasstudied in rats. The gel fraction isolated from the seeds showed significant cytoprotective effect due to the anti-secretory action and by the effects on mucosal glycoproteins [15]. On the other hand, Psyllium has been reported in humans for the treatment of different gastrointestinal disorders such as constipation, diarrhea, irritable bowel syndrome, inflammatory bowel diasease and ulcerative colits [16].

In another in vitro study, it was demonstrated that certain components, such as guar, psyllium, and fenugreek, contribute to the formation of a viscous gel in an acidic gastric environment [11]. This gel may help protect the mucosa from the harmful effects of acidity [11]. Furthermore, recent in vitro studies suggest that during simulated digestion, the mucus retains its structural integrity due to its resistance to acidic conditions, ensuring continued protection throughout the digestive process [17]. The enhanced effectiveness of these products may be attributed to the anti-inflammatory properties of their plant-based extracts (Promugel®), along with the acid-neutralizing effects of *calcium carbonate* and *magnesium hydroxide*, which help increase gastric pH levels [12]. Together, these studies and the present one highlight the potent effects of natural product combinations, which offer potential anti-inflammatory, antioxidant, and cytoprotective benefits [11,12].

4. CONCLUSIONS

Based on our results, *Gastrik pet*® effectively protects gastric cells from cytotoxicity induced by acidic pH, showing a significant reversion of pH-induced cytotoxicity. On the contrary, Sucralfate did not exert the same gastroprotective effect in this model, when used at the same concentrations of *Gastrik pet*®. In summary, *Gastrik pet*® demonstrated higher efficacy than Sucralfate, and shows promise as a candidate for further in vivo testing in animal models.

CONFLICT OF INTEREST

The authors declare to have any conflict of interest.

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