

Anti-Inflammatory Activity of Gastrik Pet (Candioli Srl) on Gastric Epithelium Cells

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Abstract

Gastritis and mucosal erosions are common clinical challenges in veterinary medicine. While conventional treatments manage acidity, there is a growing demand for nutraceuticals that directly address gastric inflammation. This study investigated the anti-inflammatory potential of the multi-component formulation, using an *in vitro* model of human gastric epithelial cells (AGS). Cell viability was first assessed via MTT assay to identify non-cytotoxic dosages. Subsequently, AGS cells were challenged with Tumor Necrosis Factor-alpha (TNF- α) to simulate inflammatory stress. The efficacy of this formulation was quantified by measuring Interleukin-8 (IL-8) secretion via ELISA. Gastrik Pet demonstrated no cytotoxicity at concentrations up to 10 mg/ml. Following TNF- α stimulation, the product significantly reduced IL-8 release across all tested non-toxic concentrations (10, 5, and 2.5 mg/ml; $p < 0.0001$). This product exerts potent anti-inflammatory effects *in vitro*, providing a scientific rationale for its use in managing inflammatory gastric disorders in pets.

Keywords: Pet, Natural products, Gastritis, Inflammation, Interleukin

Introduction

Gastritis and gastric mucosal erosion are prevalent clinical challenges in veterinary medicine such as canine and feline patients (Smyth & Toal, 2016). The gastric mucosal barrier is a defense system composed of pre-epithelial (mucus-bicarbonate layer), epithelial (tight junctions and regenerative capacity), and post-epithelial (mucosal blood flow) components. Chronic or acute injury due to dietary indiscretion and drug administration (such as NSAIDs) can disrupt this equilibrium, leading to an unregulated inflammatory response (Simpson, 2003; Smyth & Toal, 2016). The

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gastric epithelium responds to injury by secreting a variety of signaling molecules. Among these, Interleukin-8 (IL-8) stands out as a key chemoattractant (Aihara *et al.*, 2004; Sartor, 2006). In the presence of pro-inflammatory stimuli such as Tumor Necrosis Factor-alpha (TNF- α), gastric epithelial cells significantly upregulate IL-8 production, which in turn promotes leukocyte infiltration. This process, while part of the innate immune response, often leads to secondary tissue damage. While conventional treatments such as proton pump inhibitors and H₂-receptor antagonists are usually used to manage gastric acidity, there is a growing clinical demand for integrative solutions that directly address the inflammatory component and support the structural integrity of the mucosa. Nutraceuticals with proven anti-inflammatory properties offer a promising adjunct therapy to stabilize the gastric environment (Beyen, 2017). Gastrik Pet (Candioli srl) is a multi-component formulation that has been yet studied “*in vitro*” (Lonigro *et al.*, 2024, 2025) specifically developed to support gastric health in pets. This study utilizes the AGS human gastric epithelial cell line *in vitro* model for studying gastric pathophysiology (Yamaoka, 2010) to investigate the anti-inflammatory potential of the product. The objective is to quantify the efficacy of Gastrik Pet (Candioli srl) in modulating IL-8 secretion following a TNF- α challenge, providing a scientific basis for its use in the management of inflammatory gastric disorders. Specifically, the “*in vitro*” capacity of this product, at three different non-toxic concentrations, to reduce the production of the pro-inflammatory cytokine IL-8 has been assessed.

Materials and Methods

Cell Line and Growth Conditions

The experiment was conducted using the AGS human gastric adenocarcinoma cell line, with epithelial morphology (ATCC cat. No CRL-1739), provided by the Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna (IZSLER, Italy). AGS cells were selected as an established *in vitro* model for assessing gastric epithelial inflammatory responses. The cells were maintained in F12K medium supplemented with 10% Fetal Bovine Serum (FBS), 1% L-glutamine, and 1% penicillin/streptomycin, in a humidified atmosphere at 37°C and 5% CO₂.

Product Preparation

Gastrik Pet (Candioli srl) (**Table 1**) was pulverized using a mortar, solubilized in Phosphate-Buffered Saline (PBS), and sonicated to

ensure maximum dissolution. Subsequently, the solution was filtered using a 0.22 µm filter to guarantee sterility.

Table 1. Ingredients of the product tested in the study Gastrik pet® (Candioli Pharma srl).

Ingredient	%	mg/2g tablet
Microcrystalline cellulose	38.5	770
Yeasts, inactivated	13.4	668
Palatability enhancer (Optimizador Uranus)	Sodium pyrophosphate	5.7
	Yeasts, inactivated	3
	Lupin protein meal	1.21
	Sunflower oil	0.09
L-threonine	6.3	126
Calcium carbonate	5	100
Magnesium hydroxide	3.8	76
Methyl sulphonyl methane	3.1	62
<i>Thea sinensis</i> L. = <i>Camellia thea</i> Link. = <i>Camellia sinensis</i> (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 (<i>Aloe vera</i>)	0.6	12
<i>Glycyrrhiza glabra</i> L.: Licorice extract (wb)	0.6	12
<i>Promugel</i> ®: Guar meal, Psyllium – <i>Plantago Ovata</i> L. powder cuticle, <i>Trigonella foenum-graecum</i> L. fenugreek extract	10.7	214

Cytotoxicity Test (MTT Assay)

AGS cells were seeded in a 96-well multiwall plate at a density of 103 cells/well in complete medium. The following day, the medium was removed and replaced with 100 µl of F12K supplemented with 1% FBS. Treatment was performed via 1:2 serial dilutions, starting from a concentration of 80 mg/ml. The range of concentrations used in the test accounted for both the solubility limit of the product and the theoretical bioavailability concentrations following oral intake. Untreated cells (CTR) were included as a negative control. The cells were incubated with the product for 24 hours at 37°C. Following incubation, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reagent was added at a final concentration of 0.5 mg/ml, and the plate was incubated for 2 hours. After incubation, the reduced MTT crystals were solubilized by removing the medium and

adding 100 µl of isopropanol. Absorbance was measured using an Infinite M NANO + plate reader (Tecan) at 595 nm. Cell viability is expressed as a percentage of absorbance at 595 nm relative to the untreated cells (100%).

Anti-inflammatory Activity

AGS cells were seeded in a 48-well multiwall plate at a density of 3×10⁴ cells per well in complete medium. The following day, the medium was removed and replaced with 500 µl of F12K + 1% FBS. To simulate inflammatory conditions, cells were incubated with TNF-α and treated with Gastrik Pet (Candioli srl). The dosages of Gastrik Pet (Candioli srl) selected for the treatment (**Table 2**) were determined based on theoretical bioavailability, following oral administration of the maximum daily dose for a 10 kg animal, in conjunction with the results of the cytotoxicity test.

Table 2. Concentrations of Gastrik Pet (Candioli srl) utilized in the anti-inflammatory activity assay.

Product	Concentration 1 (mg/ml)	Concentration 2 (mg/ml)	Concentration 3 (mg/ml)
Gastrik Pet (Candioli srl)	10	5	2.5

Untreated cells were included as a negative control (CTR), while cells treated solely with TNF-α served as the positive control. The treatments were incubated for 24 hours, and each experimental condition was analyzed in triplicate. Following incubation, the supernatant was collected and utilized to quantify the pro-inflammatory cytokine IL-8. Cytokine levels were quantified using a specific ELISA kit, in strict accordance with the manufacturer's protocol.

Statistical Analysis

All experiments were performed in three independent experimental runs, with each experimental condition assessed in triplicate. Data are presented as mean ± standard deviation (SD). Statistical differences among experimental groups were evaluated using one-way analysis of variance (ANOVA). A p-value < 0.05 was considered statistically significant.

Results and Discussion

Cytotoxicity Test (MTT Assay)

The cytotoxicity assay was performed to select non-cytotoxic *in vitro* concentrations for the subsequent anti-inflammatory assay. AGS cells were treated with ten serial dilutions of Gastrik Pet (Candioli srl) for 24 hours, and cell viability was assessed via the MTT assay. The results are presented in (Figure 1). Notably, the 80 mg/ml dose exerted a potent cytotoxic effect on the cells. Furthermore, a slight but significant cytotoxic effect was observed following treatment with the product at concentrations of 40 mg/ml and 20 mg/ml. Consequently, 10 mg/ml was identified and selected as the highest non-cytotoxic dose.

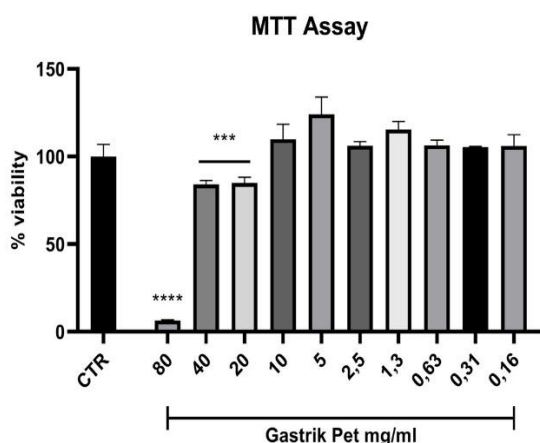


Figure 1. Cytotoxicity Assay.

AGS cells were treated for 24 hours with ten serial dilutions of the product Gastrik Pet (Candioli srl). Following treatment, cell viability was assessed via MTT assay. Results are expressed as the percentage (%) of cell viability relative to the untreated control (CTR). **** p-value < 0.0001, *** p-value < 0.0003 (One-way ANOVA).

Anti-inflammatory Activity

To evaluate the anti-inflammatory properties of the product, an inflamed AGS cell model was established by treating the cells with TNF- α , thereby simulating a condition of inflammatory stress. The concentrations of Gastrik Pet (Candioli srl) utilized for this assay are detailed in (Table 2). The results presented in (Figure 2) demonstrate that Gastrik Pet (Candioli srl) exerts a statistically significant effect in reducing the levels of IL-8 released by the gastric epithelial cells. This anti-inflammatory efficacy was consistently observed across all tested dosages.

Gastrik Pet (Candioli srl) demonstrated statistically significant efficacy in reducing inflammation in the *in vitro* gastric epithelium model at all three tested concentrations: 10, 5, and 2.5 mg/ml ($p < 0.0001$).

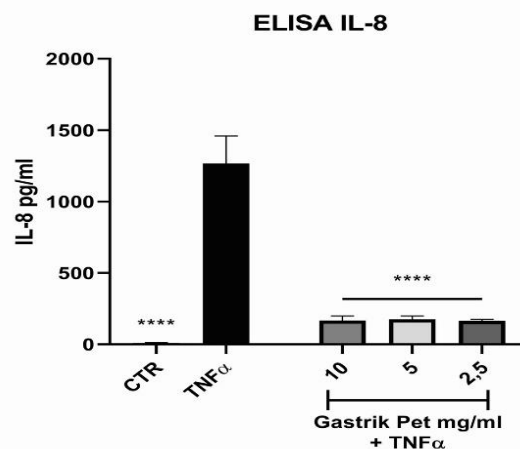


Figure 2. Anti-inflammatory activity.

AGS cells stimulated with TNF- α and treated with Gastrik Pet (Candioli srl) for 24 hours at three different concentrations. CTR: non-inflamed control; TNF- α : inflamed control. **** p-value < 0.0001 (One-way ANOVA).

A potentially relevant contribution to the anti-inflammatory activity observed in the present study may derive from Promugel®, the multi-component botanical blend included in Gastrik Pet. Promugel® combines guar meal, *Plantago ovata* (psyllium) seed husk powder and *Trigonella foenum-graecum* extract, thereby providing a complex combination of phytochemicals and functional polysaccharides with potentially complementary biological activities (Table 1).

Among these components, *T. foenum-graecum* is particularly relevant from a phytochemical perspective. Fenugreek contains steroidal saponins and sapogenins, including diosgenin, as well as trigonelline, flavonoids and other polyphenolic compounds. Diosgenin has been extensively investigated for its anti-inflammatory properties and has been reported to interfere with NF- κ B-dependent signalling and the downstream expression of inflammatory mediators (Christella, 2025). Thus, fenugreek-derived constituents may represent some of the most plausible directly active phytochemicals within the Promugel® blend.

The other components of Promugel® may provide complementary effects. *Plantago ovata* husk is particularly rich in complex mucilage polysaccharides, predominantly arabinoxylans, and also contains minor phenolic and flavonoid constituents. Psyllium-derived components have been associated with gastrointestinal protection, modulation of inflammatory responses and support of mucosal homeostasis. Guar is characterized predominantly by galactomannan polysaccharides and, as a soluble fibre component, may additionally contribute to the gastrointestinal protective properties of the blend (Laurindo *et al.*, 2023).

Taken together, these literature data suggest that Promugel® should be considered as a multi-component botanical system in which the more directly anti-inflammatory phytochemicals of *T. foenum-graecum* may act in combination with the functional polysaccharide fractions of psyllium and guar. Such

complementary activities could plausibly contribute to the marked reduction in IL-8 secretion observed following TNF- α stimulation. Nevertheless, as the present experiments were performed using the complete Gastrik Pet formulation rather than isolated Promugel® or its individual ingredients, the relative contribution of each constituent cannot be determined from the present study and possible additive or synergistic effects should be considered.

Conclusion

Gastrik Pet (Candioli srl), at all three tested concentrations, demonstrated potent anti-inflammatory properties, as evidenced by its high efficacy in mitigating the inflammatory effects induced by TNF- α in AGS gastric epithelial cells. These findings provide a potentially scientific rationale for using this multi-component formulation to support mucosal integrity and downregulate pro-inflammatory cytokines in inflammatory gastric disorders. The observed efficacy is likely driven by the synergistic biological activity between the buffering components and the botanical complexes within the formulation. To fully establish its clinical utility in veterinary medicine, future research should focus on prospective *in vivo* trials in canine and feline patients suffering from acute or chronic gastritis, alongside molecular investigations into upstream signaling pathways, tight-junction preservation, and local bioavailability.

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Conflict of interest: None

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Ethics statement: This study was performed entirely *in vitro* using the commercial AGS human gastric epithelial cell line. No live animals or human subjects were used; therefore, institutional ethical approval reference numbers are not applicable.

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