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Evaluation of the effects of Propomin® on gastrin and motilin levels in healthy dogs

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ABSTRACT

Gastritis is a common differential diagnosis in cases of anorexia and vomiting in dogs. This condition may occur secondary to infectious diseases, drug administration, or stress. This study aimed to investigate the effects of Propomin® administration, a herbal formulation containing licorice, peppermint oil, ginger root, chamomile extract, and slippery elm extract, on serum gastrin and motilin concentrations in dogs. This study was conducted on 23 dogs in total. The dogs used in this study (n:23) were between 2-10 years of age, 3-15 kg of body weight. These dogs were divided into study (Propomin®) (n:13) and control group (n:10). All dogs underwent a clinical examination. Propomin® and placebo pills were applied for 3 days by using oral route. All dogs were fed with the same commercial wet food (Nutri Grain Free®, Germany) before blood collection. Blood samples collection and glucose measurement were carried out at specified time intervals. Gastrin and motilin hormones level belong to some repeated measurement time were statistically significantly different between the study and control groups ($p<0.05$, 0.01). In the Roc analysis results for motilin, statistically significant results were obtained at 0, 60, 120, 180, and 240 minutes ($p<0.05$). Sensitivity was determined as 100% and 92.31% at the 0th and 180th minutes, respectively. Specificity values were determined as 100% (60th minute) and 90% (other minutes) at the 60th, 120th, 120th, 180th and 240th minutes, respectively. In the Roc analysis results for Gastrin, statistically significant results were obtained in the 30th, 60th and 120th minutes. It was determined that gastrin sensitivity and specificity values varied between 46.15% and 76.92%; 90% and 100%, respectively. It is thought that the limited increase in gastrin hormone levels caused by Propomin® may indicate a possible suppression of HCl acid secretion in parietal cells similar to the effect caused by proton pump inhibitors. On the other hand, the decrease in motilin levels as a total effect of these ingredients strengthens the idea that they produce a spasmolytic effect.

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Introduction

Gastritis or gastric ulcer is one of the most frequently observed gastrointestinal diseases in dogs. Many factors such as viral infections, non-steroidal anti-inflammatory drug (NSAID) use, stress, *Helicobacter pylori* infection play an important role in the formation of the disease (Amorim et al., 2016). On the other hand, gastric and duodenal hypomotility are also reported to play a role in the formation of the disease. Antral hypomotility, pyloric sphincter abnormalities contribute to the formation of gastric ulcers by causing duodenogastric reflux (Moore et al., 1986).

Motilin is an intestinal hormone mainly produced by enteroendocrine cells in the duodenum and also plays a role in the control of gastrointestinal motility. In that fact, motilin concentration has been considered in studies regarding gastrointestinal movements. Motilin can vary depending on several factors. Changes such as fasting, low duodenal pH level, and gastric pH can affect motilin concentrations (Ogawa et al., 2012). It has been

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reported that plasma motilin concentration increases during the interdigestive phase in accordance with the cyclic interdigestive contractions of the stomach, is suppressed by food intake, and interdigestive contractions can be induced by external application. On the other hand, it has also been reported that it can be secreted intermittently during the interdigestive period (Itoh et al., 1978). In a study conducted by Matsuanaga et al. (1994), it was determined that endogenous motilin concentration was low in dogs with gastric acidification and phase III contraction inhibition (Matsunaga et al., 1994).

Gastric acid production occurs as a result of gastrin production via vagal stimulation and histamine secreted by enterochromaffin cells stimulating the periatal cells in the stomach. (Mordecai et al., 2011). Gastrin hormone may vary depending on factors such as decreased renal clearance, liver problems, chronic enteritis, gastrinoma, and use of gastric acid suppressant drugs, parasympathomimetic or parasympatholytic drugs, in addition to diseases associated with gastritis (Heilmann et al., 2017; Kovacs et al., 1989; Mazaki-Tovi et al., 2012; Thomas et al., 1979).

Many studies has been reported regarding the use of licorice, peppermint oil, ginger root, chamomilla, slippery elm extracts alone, their anti-ulcer, anti-inflammatory, anti-helicobacter pylori and antibacterial and motility regulating effects (Aly et al., 2005; Beesley et al., 1996; Kwon et al., 2020). However, there are no any knowledge in literature on the gastroprotective effects of these ingredients any studies expresses on their combined application.

Glycyrrhizin in licorice root is converted to glycyrrhizic acid and subsequently glycyrrhetinic acid in the stomach. (Kwon et al., 2020). At the same time, in the stomach, carbenoxol, which is composed of glyceric acid, inhibits the hormone gastrin and increases local concentrations of prostaglandins, which promote the increase and repair of mucus (Ishii & Fujii, 1982; van Marle et al., 1981). Some active constituents of ginger root have been reported to increase muscle activity in the digestive tract, thus stimulating digestion and absorption while relieving constipation and bloating (Wu et. al., 2020). Ginger root has also been found to stimulate bile secretion, intestinal lipase, trypsin, chymotrypsin, amylase, sucrose and maltase activities in rats and 6 and 10 gingerols are mainly responsible for this activity (Platel & Srinivasan, 2000; Platel & Srinivasan, 1996). On the other hand, activation of pancreatic secretions may have a negative stimulatory effect on gastrin release (Howe, 1966). Studies on the gastroprotective effects of chamomila extracts have focused on gastroprotective effects rather than direct effects on gastrin levels.

In this study, Propomin® was considered for its potential gastroprotective, prokinetic, and gastrointestinal spasm-relieving effects, which could help reduce polypharmacy in gastrointestinal disorders. The aim of the study was to evaluate the effects of Propomin® constituents (licorice, peppermint oil, ginger root, chamomilla extract, and slippery elm extract) on gastrin and motilin hormones, and to investigate their potential impact on gastric acid secretion and motility using dogs as the experimental model.

Material and Methods

Ethics Statement

The study protocol was approved by the Bursa Uludağ University Local Ethics Committee for Animal Experiments (HADYEK-2023/12-07, dated November 7, 2023).

Material selection and exclusion criteria

The dogs used in this study (n=23) were between 2–10 years of age, weighing 3–15 kg, with equal sex distribution. Clinical examinations were performed in the study (n=13) and control (n=10) groups before administration of Propomin® (Cureeffect Animal Health, Turkey) or placebo capsules. Propomin® capsules contained the active herbal formulation, whereas placebo capsules administered to the control group consisted of empty shells without any content. Propomin® is a multi-component herbal formulation in capsule form, with the following composition per capsule: licorice extract 10,000 mg, peppermint extract 12,500 mg, ginger root extract 5,000 mg, chamomilla extract 7,500 mg, and bentonite q.s. All components were in powdered form within the capsules. Clinical examination included assessment of mucous membranes, body temperature (°C), heart rate (HR), respiratory rate (RR), abdominal tenderness, gastrointestinal and lung auscultation, lymph nodes, capillary refill time, and clinical dehydration assessment. Subjects with gastrointestinal problems, anorexia, vomiting, or abnormal clinical findings were excluded, and only dogs within normal reference limits were included in the study.

Study design

Prior to blood sampling for blood glucose and hormone (gastrin and motilin) analyses, dogs in both the study and control groups were fasted for 12 hours with water withheld in a separate compartment. All dogs were fed the same commercial wet food (Nutri Grain Free®, Germany) for 7 days before the experiment to minimize dietary variation. On the study day, the same diet

dietary variation. On the study day, the same diet (protein 10.50%, fat 6.20%, crude ash 2.30%, crude fiber 0.40%, moisture 77%) was administered at a dose of 20 g/kg to both groups. The study included two groups. Dogs were randomly allocated to the treatment or control group using a computer-generated random number sequence to avoid selection bias. The sample size was determined based on previous studies investigating motilin and gastrin physiology in dogs. In the literature, small to moderate group sizes are commonly used in similar physiological research (e.g., Itoh et al., 12 healthy dogs; Strunz et al., 6 dogs; Seim-Wikse et al., 15 patients and 7 control dogs). Accordingly, the sample size in our study (treatment group n=13; control group n=10) was selected in alignment with prior research. As this study was exploratory in nature, this sample size was considered sufficient to obtain preliminary physiological data. Group 1 (Study Group): They were recruited from cases without any health problems. They were given Propomin® (1 solid capsule per 10 kg body weight) for 3 days. On the study day, 20 g/kg dose of wet food was fed. Group 2 (Control Group): Subjects in this group ingested empty capsules (1 solid capsule per 10 kg body weight) for 3 days.

Collection of blood samples

Blood samples for serum and plasma were collected at 0- minutes (pre-prandial) and at 30-, 60-, 120-, 180-, and 240- minutes post-prandially. At each sampling point, blood glucose concentrations were measured immediately using a portable glucometer (Fora Comfort Check®-G40).

Blood samples used in this study were collected from dogs V. Antebracihi in sterile empty tubes (Vacusera 5 ml) each time. For plasma samples, samples were collected in EDTA-filled tubes (Vacusera 2 ml). Glucose was measured with a glucose meter (FORA COMFORT Check G40, Istanbul) at each blood collection time. Serum and plasma samples were spun for 5 minutes with a centrifuge device (Elektro-Mag M415M®) at 3000 rpm. The samples were stored at -20 °C in 3 ml cryotubes with the addition of Aprotinin (0.6 TIU/ml) (Phoneix Pharmaceyticals Inc®, USA) at the rate of 0.1 ml per 1 ml. ELISA assays were performed 7 days after the samples were collected.

Performing ELISA tests. For the measurement of canine-specific gastrin (Standard Curve Range, 1 pg/mL-120 pg/mL) and canine-specific motilin concentrations (Standard curve range, 10 pg/mL-1200 pg/mL), commercial dog-specific ELISA kits were used ((Shanghai Coon Koon Biotech Co. Ltd® ,China) Canine Gastrin Catalog no: CK-bio-18832 and Canine Motilin Catalog no: CK-bio-22512). Serum samples were analyzed by double antibody sandwich ELISA technique using an Epoch microplate reader (BioTek Instruments, Winooski, VT, USA). The highest and lowest standard Gastrin Hormone concentration ranges that can be measured in serum were between 18.38 pg/mL-14.95 pg/mL. In the measurement of Canine motilin values, the highest and lowest standard concentration ranges were between 1.90 pg/mL-1.41 pg/mL. No dilution of serum samples was required when performing the ELISA kit procedure. As a result of Canine gastrin hormone measurement, the R2 value of the standard figure (% accuracy of the study) was 1 (100%); R2 value of the Canine motilin standard figure was 1 (100%). The CV value for the test (n = 7) was calculated as 4.68% for motilin and 3.8% for gastrin (n = 7).

Statistical Analysis

Normality of all variables at each time point was assessed using the Shapiro–Wilk test. All datasets met the normality assumption except for the motilin values in the control group. For repeated measures, each time point was evaluated separately. Normally distributed data were analyzed using repeated-measures ANOVA, whereas non-normally distributed data were analyzed using ANOVA on ranks. Paired t-tests were applied for within-group comparisons across repeated time points due to the exploratory nature of the study design and the limited sample size, consistent with previous physiological research in dogs. For comparisons between independent groups, independent-samples t-tests were used for normally distributed variables. Descriptive statistics are presented as mean ± SE for parametric data. To evaluate the effect of Propomin® administration, ROC curve analysis was performed to determine cut-off values for gastrin and motilin. variables with statistically significant area under the curve (AUC) values were further analyzed using Youden's J index to identify optimal thresholds. The ROC analysis was also interpreted to explore the potential diagnostic relevance of changes in Gastrin and Motilin concentrations as indicators of gastric physiological responses to herbal formulations.

All statistical analyses were conducted using IBM SPSS v22 (IBM Corp., Armonk, NY, USA) and MedCalc v19.1.3 (MedCalc Software Ltd., Ostend, Belgium). A p-value of < 0.05 was considered statistically significant.

Results

The dogs belonging to the study group were arranged as 6 females-7 males and the dogs belonging to the control group were arranged as 5 males-5 females. Clinical examination findings are shown in Table 1. After the clinical examinations, the values of all subjects were found to be within the reference limits. According to the information received from the caregivers, none of the dogs had any complaints of chronic diseases such

Table I Clinical examination findings of dogs participating in this study, study and control groups, mean ± SE values.

Vital Parameters	Control group	Study group	Referance values (Sarıtaş, 2013)
Body Temperature (°C)	38.54±0.202	38.52±0.215	37.5-39.3
Heart Rate beats/min	165.6±10.38	154.76±8.9	80-160
Respiration/min	48.8±3.41	46.61±1.8	10-60
Lymph Nodes	N	N	-
Mucous membranes	N	N	-
CFT	N	N	3-5 sn
Lung Auscultation	N	N	-
Gastrointestinal Auscultation/Sensitivity	N	N	-

Table 2. Mean and SE (±) values of blood Glucose. Gastrin and motilin levels according to blood collection times

	Blood collection time					
Gastrin-Motilin	**0**	**30**	**60**	**120**	**180**	**240**
Gastrin C. pg/mL	16.58 ± 0.48	15.38 ± 0.34[a]	14.95 ± 0.48[a]	15.29 ± 0.64[a]	15.32 ± 0.66	15.74 ± 0.91[a]
Gastrin S. pg/mL	16.99 ± 0.89	18.09 ± 0.75[a]	16.68 ± 0.52[a]	18.13 ± 0.87[a]	16.75 ± 0.83	18.38 ± 1.04[a]
Motilin C. pg/mL	1.87 ± 0.11[a]	1.84 ± 0.09[a]	1.90 ± 0.05[a]	1.83 ± 0.05[a*]	1.87 ± 0.08[a]	1.89 ± 0.11[a]
Motilin S. pg/mL	1.45 ± 0.04[a]	1.60 ± 0.07[a]	1.50 ± 0.05[a]	1.53 ± 0.04[a*]	1.41 ± 0.04[a]	1.51 ± 0.06[a]
Glucose C. mg/dL	71.6 ± 4.8	72.7 ± 4.9	76.1 ± 4.9	78.5 ± 3.8[a]	85.2 ± 5.7[a]	77.9 ± 2.94
Glucose S. mg/dL	73.9 ± 3.7	76.7 ± 2.8	77.1 ± 2.5	66.9 ± 1.6[a]	73.8 ± 2.04[a]	73.2 ± 2.84

a. Same letters indicate statistical difference between paired samples in the same column. paired t test. p<0.05; *. p<0.01; C; control S; Study

as vomiting, gastrointestinal disorders, cachexia, diarrhea, obesity, polyphagia, polyuria, polydipsia.

Gastrin hormone levels were statistically different between the study and control groups at 30, 60, 120, 240 minutes. Regarding motilin hormone levels, there was a statistical difference between the study and control groups at all blood collection times (p<0.05, 0.01). There was no difference in blood collection times for repeated measurements in both groups (Table 2). While blood glucose levels were stable in both groups, there was no statistical difference in blood collection times. However, there was a statistical difference between the study and control groups at 120 and 180 minutes (P<0.05) (Table 2).

Statistically significant results were obtained in ROC analysis results for motilin at 0, 60, 120, 180, 240 minutes (Table 3 and Figure 1). At 0 and 180 minutes, the sensitivity levels were 100% and 92.31%. At 60, 120, 180 and 240 minutes, the specificity values were 100% (60th minute) and 90% (other minutes), respectively (Table 3 and Figure 1). ROC analysis results for gastrin showed statistically significant results at 30, 60 and 120 minutes (Table 3 and Figure 2). Sensitivity values for gastrin ranged between 46.15% and 76.92%, while specificity values ranged between 90% and 100%. Cut off values, 95% CI, AUC, sensitivity, specificity and Youden J index values of ROC analysis results for motilin and gastrin are given in Table 3 (Table 3 and Figure 2).

Table 3. ROC analysis results for motilin and gastrin

Motilin pg/mL	Cut-off Value	SE	95% CI	AUC	Sensitivity	Specificity	Youden J Index	P
0	≤1.742	0.089	0.66 to 0.97	0.869	100	70	0.7	<0.0001
60	≤1.603	0.044	0.76 to 0.99	0.942	76.92	100	0.769	<0.0001
120	≤1.651	0.059	0.72 to 0.99	0.915	84.62	90.0	0.746	<0.0001
180	≤1.592	0.043	0.77 to 0.99	0.946	92.31	90.0	0.823	<0.0001
240	≤1.627	0.069	0.68 to 0.98	0.885	76.92	90.0	0.669	<0.0001
Gastrin pg/mL								
30	>16.114	0.091	0.62 to 0.96	0.838	76.92	90.0	0.669	0.0002
60	>16.625	0.101	0.54 to 0.91	0.765	46.15	100.0	0.461	0.0089
120	>17.335	0.102	0.57 to 0.93	0.792	61.54	100.0	0.615	0.0043

SE: Standart error. CI: Confidental Interval. AUC: Area Under Curve

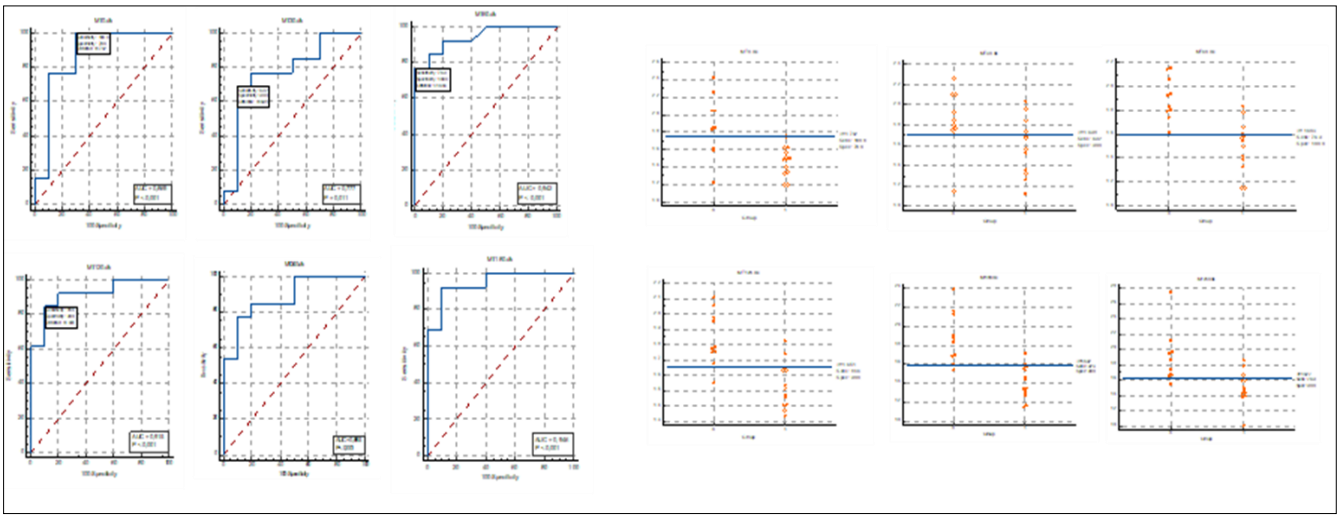
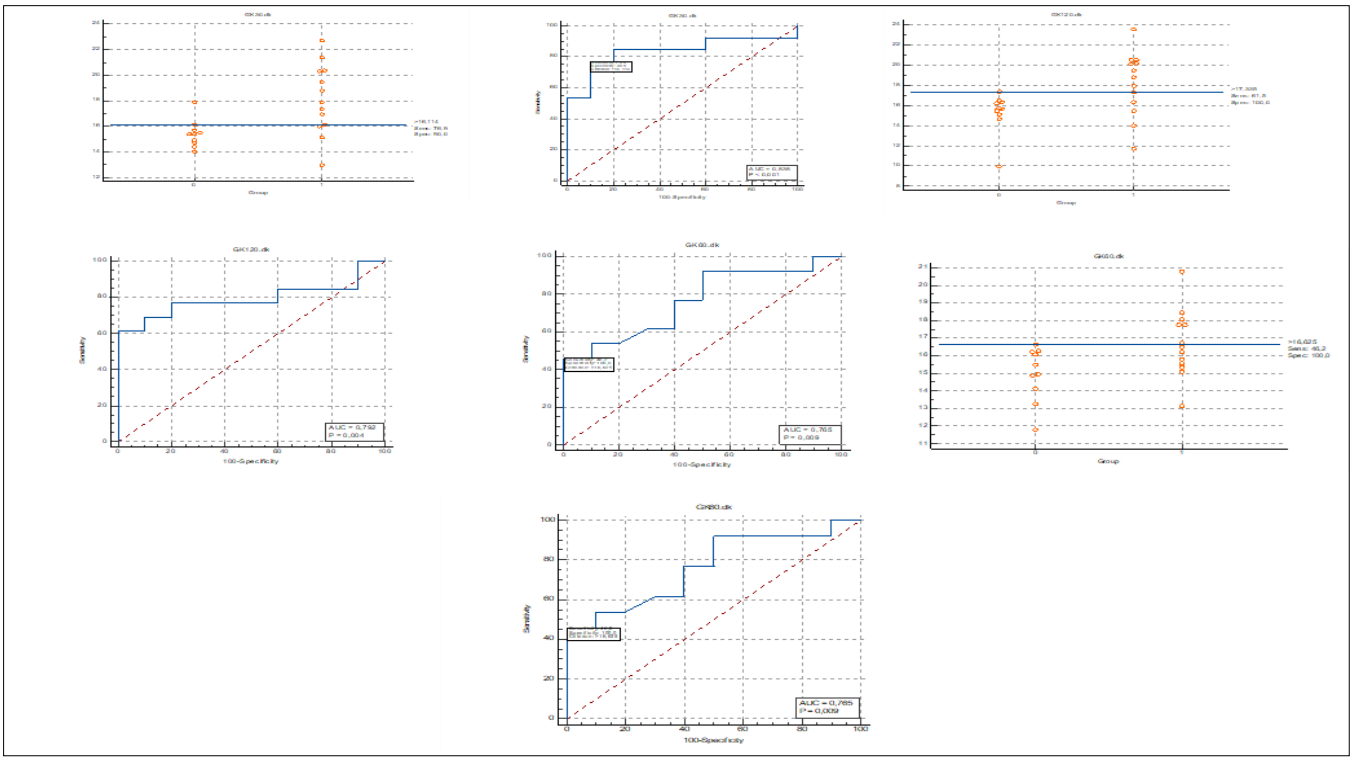


Figure 1. AUC and dot plots for motilin, (cut off; pg/ml)

Discussion

Propomin® is a multi-component herbal formulation containing licorice, ginger root, chamomile, peppermint oil, and slippery elm. Therefore, the literature discussed in this section refers to previously reported pharmacological effects of these individual constituents. However, the present study did not investigate each component separately. Thus, the cited studies provide supportive mechanistic insights into potential actions of the constituents within Propomin®, rather than direct, component-specific evidence from the current experimental design.

Licorice, one of the Propomin® ingredients, is an herbal treatment option that finds many uses in alternative medicine. Among these, gastrointestinal disorders are in the first place. Ishii and Fujii (1982) reported an increase in serum gastrin concentration in rats and a decrease in gastrin concentration in dogs after administration of FM100, a fraction of licorice root extract, at a dose of 800 mg/kg (Ishii & Fujii, 1982). Again, an important effect of this plant extract is to increase the eradication of *H. Pylori*. By blocking the enzyme dihydrofolate reductase and DNA gyrase, it produces an antibacterial and anti-adhesion effect against *H. Pylori* (Kwon et al., 2020). Ginger root, another ingredient of Propomin® used in this study, has been shown to increase pancreatic and intestinal lipase secretion in animals (Platel & Srinivasan, 2000; Platel & Srinivasan, 1996). Some active constituents of ginger root have been reported to increase muscle activity in



Figüre 2. AUC and dot plots for gastrin, (cut-off; pg/ml)

in the digestive tract, thus stimulating digestion and absorption while relieving constipation and bloating (Wu et al., 2020). Ginger root has also been found to stimulate bile secretion, intestinal lipase, trypsin, chymotrypsin, amylase, sucrose and maltase activities in rats and 6- and 10- gingerols are mainly responsible for this activity (Platel & Srinivasan, 2000; Platel & Srinivasan, 1996). On the other hand, activation of pancreatic secretions may have a negative stimulatory effect on gastrin release (Howe, 1966). These findings support the traditional use of ginger as a digestive stimulant, hence the effects of the methanolic extract of ginger on gastric acid secretion in the aforementioned study. Along these lines, Obia and Udeh (2023) reported that in a study using ginger plant extracts, gastric pH increased to 6.7 at 45 minutes after oral extract ingestion in their study group, while it remained 5.95 in the control group. Although bicarbonate levels were also reported to decrease in the study group, it should be noted that bicarbonate was not evaluated in the present study (Udeh & Obia, 2023). In a study by Nanjundaiah et al. (2011), it is stated that ginger plant extracts block gastric acid release in relation to H+, K+-ATPase action and provide gastric protection with anti-H.pylori and anti-oxidant activity (Nanjundaiah & Dharmesh, 2011). Chamomilla preparations inhibited pepsin release and thus showed antiulcer effect. A study in dogs reported an increase in pepsin out-put after gastrin release stimulated by Bombesin (Hirschowitz & Molina, 1983). In the light of these evaluations, it can be considered that chamomilla extracts suppress gastrin release based on its effect on pepsin. The literature indicates that chamomilla, ginger, and licorice extracts have potential effects on gastrin. On the other hand, the protective effects of slippery elm on the luminal mucosa and peppermint on the gastrointestinal motility are more pronounced. In this study, although there was no significant difference in the initial period of blood sampling times in the study group, it was found that gastrin levels were higher in this study group than in the control group at all times except 180 minutes ($p<0.05$). In addition, ROC analyses revealed statistically significant cut off values of gastrin levels at 30, 60 and 120 minutes and AUC values ranged between 0.76-0.83 (Table 3, Figure 2). This effect may be attributed to the licorice extract included in Propomin®, rather than the extract alone, in agreement with previously reported studies. Other extracts are thought to be more prone to the potential to suppress gastrin release than licorice. Although direct experimental evidence is not available, it is possible that the extracts in Propomin® may modulate gastric HCl secretion at the level of parietal cells, potentially influencing gastrin release. Licorice, in particular, may contribute to gastrin modulation, which could be favorable in terms of maintaining gastric homeostasis.

Many herbal products are used in gastrointestinal disorders due to their prokinetic effects. Studies on peppermint oil, for instance, have largely focused on its antispasmodic properties (Hills & Aaronson, 1991; Papathanasopoulos et al., 2013), potentially mediated through reductions in calcium influx in smooth muscles. Peppermint oil has also been reported to decrease intragastric pressure and phasic activity in the proximal stomach, particularly during fasting (Papathanasopoulos et al., 2013). Ginger root exhibits both spasmogenic and spasmolytic effects on the gastrointestinal tract, acting through cholinergic mechanisms and calcium channel antagonism (Ghayur & Gilani, 2005; Hu et al., 2011). Licorice extracts have similarly been reported to exert dual effects on gastrointestinal motility depending on concentration (Hu et al., 2011). Chamomilla extracts induce relaxation of longitudinal and circular gastric muscle fibers, reducing muscle tone (Dai et al., 2022).

In the present study, Motilin levels were significantly lower in the Propomin® group compared to controls at all time points ($p<0.05$). Considering the multiple active components of Propomin®, it is not possible to identify which extract is primarily responsible for this effect. Nonetheless, the observed decrease in motilin levels is compatible with the known spasmolytic effects of peppermint oil and the combined effects of other herbal constituents.

Glucose homeostasis was also monitored in this study to control for potential confounding effects on gastrointestinal motility, as variations in glucose levels can modulate motility patterns (Wemelle et al., 2022).

In conclusion, the modest increase in gastrin observed with Propomin® administration may suggest a potential modulatory effect on gastric HCl secretion, though further studies are needed to confirm this. Additionally, the anti-*Helicobacter pylori* activity and gastric protective properties of the herbal components may contribute to gastrin modulation. The observed decrease in motilin levels supports the notion that Propomin® exerts a spasmolytic effect. ROC analysis further suggests that changes in gastrin and motilin concentrations could serve as potential biomarkers for evaluating physiological gastric responses to herbal formulations. Collectively, these findings indicate that Propomin® may have potential as a supportive intervention in the management of gastritis and other gastric motility disorders in dogs.

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