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Overproduction of gastric mucus as a mechanism of stomach's mucosa protection during energy drinks consumption in Wistar rats

Nadmierne wytwarzanie śluzu jako mechanizm ochraniający błonę śluzową żołądka w czasie przyjmowania napojów energetycznych przez szczury rasy Wistar

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

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Summary

Aim: Energy drinks are the most consumed, socially accepted stimulants in the world. They are drunk by drivers and students, and more often also by adolescents and children. It has been noted that some adverse events are due to the stimulation of the sympathetic nervous system and include palpitations, hypertension, diuresis, nausea, vomiting, and convulsions. The health consequences of long-term consumption of energy drinks are not known, but we suppose that continuous stimulation of the body can lead to irreparable changes of tissues. In our study we investigated the impact of long-term energy drink consumption on gastric mucus production.

Material/Methods: Ten male young Wistar rats of of 190 grams +/- 64 grams of weight were positioned in two groups: A (experimental) and C (control). The animals of A group received energy drink for 8 weeks. The animals of C group drank just water. After this time the rats of both groups were decapitated and the stomach was taken for histological analysis. The slides were stained with H&E and PAS method and analyzed with Cell^D software.

Results: H&E staining indicated an increase in the thickness of the lamina propria of the glandular stomach. PAS staining indicated an increased area of mucus in the gastric glands of the stomach of the experimental group of rats.

Conclusions: Regular consumption of energy drinks can lead to changes in the mucosal lining of the stomach, which activate mucus secretion.

Keywords: **energy drink • mucosa • taurine • caffeine • protection**

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INTRODUCTION

Caffeine affects the function of the gastrointestinal system from the mouth to the anus. Large amounts of caffeine increase sweat excretion [25]. After caffeine consumption, the pressure in the lower esophageal sphincter and the contraction in the distal esophagus decrease, which is likely one of the causes of gastro-esophageal reflux [19]. Caffeine stimulates gastric acid secretion and decreases Ach-induced mucus secretion [9]. Marketed energy drinks contain from 50 mg to over 500 mg of caffeine per can or bottle [16]. Additional supplements like taurine, sugar and vitamins are popular ingredients for a number of beverages including energy drinks. Taurine has a chemical structure similar to the inhibitory neurotransmitter, gamma-aminobutyric acid (GABA), which is stored in numerous cells of the human body, such as parietal cells, neurons and smooth muscle cells. Taurine plays an important role in stabilizing cellular membranes and modulating acid secretion and gastric motility. It protects the gastric mucosa against certain lesions and regulates glucose metabolism [11,15,20]. Taurine is used as a medicament, but the safety of its concentration in energy drinks, which amounts to approximately 4.0g/L, is still being investigated [7].

Energy drinks are the most consumed, socially accepted stimulants in the world [16]. Data from the European Food Safe Authority (EFSA) suggests that energy drink consumption among the adolescent population is on the rise. The study reports that across 16 countries of the European Union, 68% of adolescents aged 10-18 years old consumed energy drinks in 2011 [3]. Routine energy drink consumption can lead to cognitive, cardiovascular, endocrine, and gastrointestinal disturbances [1]. It has been noted that some adverse events are due to the stimulation of the sympathetic nervous system and include palpitations, hypertension, diuresis, nausea, vomiting, and convulsions [3]. The health consequences of long-term consumption of energy drinks are not known, but continuous stimulation of body functions can lead to irreparable changes of tissues [12]. In our study we investigated the impact of long-term energy drink consumption on gastric mucus production.

The stomach of a rat consists of two main anatomical structures: the forestomach, which is the non-glandular part of the stomach, and the glandular part of the stomach. The forestomach is the area that receives food from the esophagus and acts as a reservoir for the incoming bolus. The glandular part contains digestive enzymes and mucus that marks the beginning of digestion controlled by the pyloric sphincter. Between these two areas, there is a low mucosal fold called the limiting ridge that separates the squamous epithelium of the forestomach from the columnar epithelium of the corpus [22]. In our study, we focused on the glandular part of the rat's stomach, which is similar to the human stomach.

It is widely known that gastric mucosa maintains structural integrity and function despite being exposed to many noxious stimuli [17]. Mucosal integrity is maintained by various defense mechanisms of which four are vital: continuous cell renewal, continuous blood flow through the mucosal microvessels, sensory innervation, and generation of prostaglandins and nitric oxide [22]. Some substances alter the balance between the level of gastric mucus production and gastric acid secretion cause gastrointestinal disturbances [14]. The ingredients of energy drinks separately modify the production of gastric acid, enzymes, bile and mucus; however, in our study we investigated if their simultaneous consumption is indifferent to gastric mucosa [7,21].

MATERIALS AND METHODS

Ten young male Wistar rats weighing 190 grams+/- 64 grams were used. Each were positioned in two groups labeled A (experimental) and C (control) with five rats per each group. Group A rats consumed an energy drink (ED) for eight weeks (average +/- 0.190 ml/g of BW per day). The energy drink was prepared from the following ingredients per 100 ml: 32 mg of caffeine, 400 mg of taurine, 20 mg of Inositol, 2 mg of Vitamin B5, 7 mg of Vitamin B3, 0.5 µg of Vitamin B12, 1.4 mg of Vitamin B6, and 10.9 g of sugar. Group C rats received only water for eight weeks. Throughout the experiment, each group was fed per day as needed. Group A and C rats were decapitated 24 hours after the end of the experiment, after which their stomachs were dissected and taken for histological analysis using two staining methods, Hematoxylin and Eosin (H&E) and Periodic Acid Schiff (PAS). Histological specimens were assessed using an optic microscope, with magnifications of 200x and 400x. The histological analysis was made using Cell^D software and the obtained data was analyzed using Statistica 10 Statsoft software.

RESULTS

H&E staining

Through H&E staining, we observed that the mucosa of the glandular stomach near the limiting ridge had shown an increased thickness of the lamina propria in the experimental group (Table 1, Fig.1), but the difference was not statistically significant as indicated by the t test, where p=0.109102.

Table 1. Statistical analysis of tested tissue

Group	Thickness of lamina propria of stomach mucosa	Mean area PAS (+) in the glandular stomach mucosa	Thickness of superficial mucus layer
Experimental (A)	374.9617±95.06346 µm	78413.2 ±19438.1 µm^2 (14% of field of view)	6.969883±2.624489 µm
Control (C)	336.4147±83.61240 µm	29424.47±7782.07 µm^2 (5% field of view)	2.360999±0.935353 µm

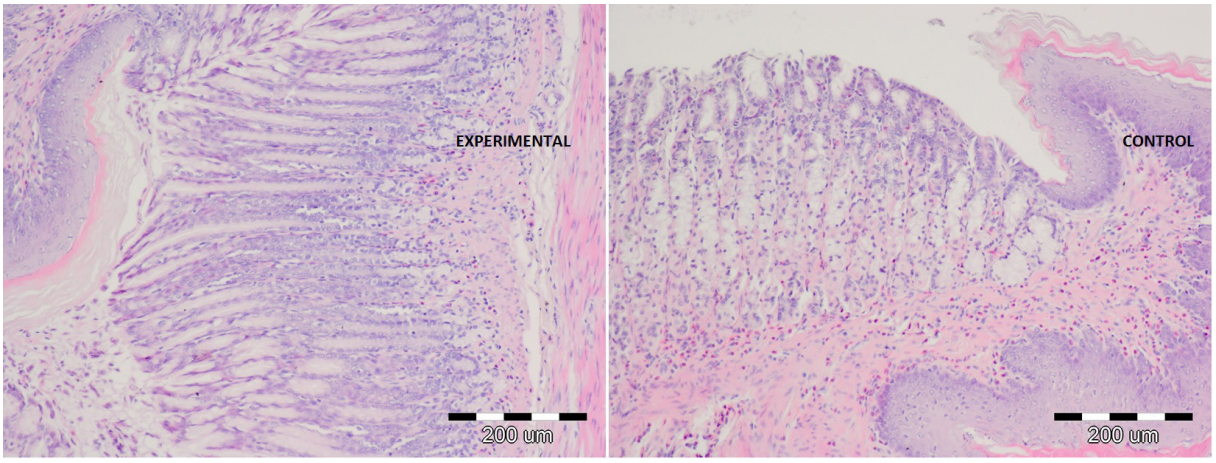

Fig. 1. Fragment of glandular stomach mucosa with limiting ridge. Slightly thicker mucosa in the experimental group than in the control group. H&E staining, Magn. ca 400 x

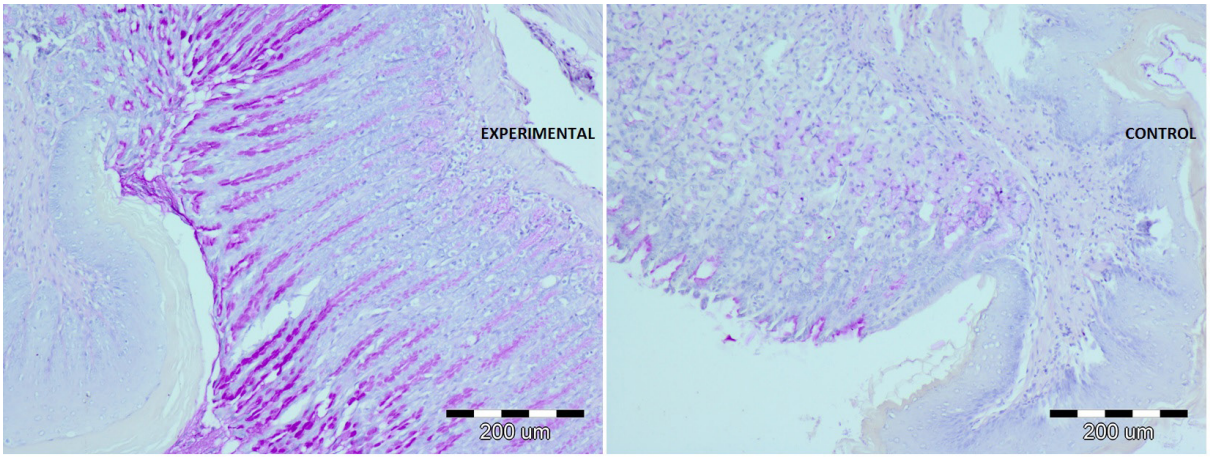

Fig. 2. Increased production of mucus in the stomach mucosa of experimental group. Fragment of glandular stomach mucosa with limiting ridge. PAS staining, Magn. ca 400 x

PAS staining

Through PAS staining, a positive reaction was noted more frequently within the area of glandular stomach mucosa including superficial mucus cells, mucus neck cells and the mucus cells located in the base region of the gastric glands of the stomach of the experimental group that in the control group (Table 1, Fig. 2). The differences were statistically significant as indicated by the t test, where p=0.015. The superficial layer of mucus, which covers the mucosa, was thicker in the experimental group than in the control group (Table 1). The differences were statistically significant, as indicated by the t test, where p<0.00001.

DISCUSSION

Consumption of energy drinks causes many different clinical adverse effects resulting from irritation of many tissues. In our study, we observed an increase of gastric mucus production after eight weeks of consuming an ED. Caffeine increases the production of gastric acid

and decreases its neutralisation in different mechanisms. The data proves that caffeine interferes with nitric oxide production leading to secondary vascular constriction and lack of blood flow to the mucosa [4]. Nitric oxide increases mucus secretion in the gastric mucosa, inhibits the activation of leukocytes within the microcirculation and inhibits the inherent release of reactive oxygen metabolites and proteases [24]. Moreover, caffeine blocks the IP3 signaling pathway in gastric mucosal cells, thus resulting in decreased mucus secretion [9]. Caffeine also inhibits cholinergic innervation of the gastrointestinal tract that decreases vagally mediated gastroprotective effects [8,27]. A study conducted in the department of pharmacology and pharmacotherapy at Semmelweis University indicated that the vagal nerve has a dual action involving both mucosal damaging and protective effects in the regulation of gastric mucosal integrity. The stimulation of the vagus nerve involves the stimulation of gastric prostaglandin, nitric oxide, and capsaicin-sensitive afferent fibers containing CGRP [8]. The alteration of vagal nerve stimulation is, therefore, one of the mechanism of gastric mucosa function disorder caused by caffeine.

The second ingredient of energy drinks, taurine also increases gastric acid production but simultaneously increases gastric mucus production and participates in the conjugation of bile acids, which may irritate the gastric mucosa [10,11]. Moreover, taurine has many biological roles in the human body [11,14]. It plays a role in neuromodulation in the central nervous system, energy production, protection against oxidation, immunomodulation and regulation of cell volume [6]. Taurine takes part in the metabolism of lipids and glucose [2,6]. In a study by Islambulchilar et al., taurine in a dose of 2g per day reduced chemotherapy-induced nausea and vomiting during therapy of acute lymphoblastic leukemia [13]. The anti-emetic properties of taurine may increase the safety of energy drink consumption and neutralize the harmful effects of other ingredients, but it is still unknown if higher than therapeutic doses of taurine are safe. Moreover, the consequence of higher than normal mucus secretion is non-acid reflux, which is also known as laryngopharyngeal reflux. Non-acid contents, such as mucus with chyme, pass through both the lower esophageal sphincter and upper esophageal sphincter and can move into the pharynx, larynx or the trachea. The complications of untreated reflux can lead to swelling of the vocal folds, ulcers on the vocal folds, worsening of asthma, emphysema, and bronchitis [26].

Another disturbance caused by energy drink consumption is hyperglycemia. Caffeine induces the absorption of glucose, which is insulin independent, and simultaneously reduces insulin sensitivity via enhanced sodium-glucose-linked transporter proteins [25,27]. Moreover, consumption of beverages with a high carbohydrate concentration (8%) reduces the gastric emptying rate [23]. Alterations in the process of glucose and electrolyte transport by the wall of the gastrointestinal tract increases the risk of dehydration and diabetes mellitus of ED drinkers [25,27].

Ingredients such as vitamins and inositol do not play an important role in the induction of adverse effects, although they may be the cause of mild diarrhea or nausea. In addition, inositol may promote female fertility, regulate glucose metabolism and it is an anti-depressant [5,18].

In summary, our study indicates that consumption of energy drinks results in microscopic changes in the gastric mucosa. Specifically, we observed an increase in mucus production, as verified by PAS staining. Increased mucus production is heavily dependent on the main substances in energy drinks, such as taurine. Further investigation is necessary to identify the long-time consequences of ED consumption by people. The addition of taurine and vitamins decreases some adverse effects of these beverages, but is not sufficient to prevent numerous irreparable pathological changes in body function. The authors indicate no potential conflicts of interest.

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