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Immunohistochemical determination of somatostatin release in gastric tissue of rats fed with a high-fat and cholesterol diet

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Abstract

This study aimed to investigate the effects of a high-fat and cholesterol diet (HFCD) on rats' gastric mucosa. In the study, a total of 16 (40-day-old Sprague Dawley) male rats were used and randomly divided into two groups (each consisted of eight rats). The rats in control group had no implementations other than normal feeding. For 10 weeks, rats in a high-fat with cholesterol diet group had daily energy amounts provided by pellet feed mixed with 65.00% butter and 2.00% cholesterol. Before beginning the study and at the end, rats live weight was recorded and their blood samples were taken for biochemical analyses. Hematoxylin and Eosin and Crossman's triple staining techniques were used to investigate the general structure of gastric tissue. The rats fed with HFCD had statistically significant increases in live weight and total cholesterol values, and were identified to have gastric tissue degeneration. The rats' gastric tissue in control group had more intense somatostatin (SST) immunoreactivity in parietal and chief cells than the HFCD group. It was determined that feeding with the HFCD has a negative effect on SST secretion in rats and hence, this may have important areas of use such as in gastric cancer treatment and preventing complications linked to gastric diseases.

Introduction

Cholesterol is a wax-like fatty material being important for the body. The body uses cholesterol to produce steroid hormones (adrenal cortex hormone and cortisone), vitamin D, and bile acid, digesting fats.[1] Cholesterol may be taken into the body through diet, or the body may synthesize the necessary cholesterol itself.[2] The liver is the organ with the highest cholesterol synthesis. Additionally, cholesterol synthesis also occurs in tissues like adrenal cortex, testis, and ovaries.[3] Cholesterol deficiency affects tissues belonging to many systems like digestive tract;[4] while, excess cholesterol leads to diseases like heart and coronary artery diseases, hypertension, and cerebral thrombosis. Cholesterol levels are affected by the lack of conscious nutrition, genetics, sport and stressful life conditions.[5] Increased blood lipid values and abnormal variations in cholesterol levels were identified to occur as a result of nutrition with a high-fat diet.[6]

The stomach is an organ in the form of a tube with an internal cavity. The stomach wall comprises four layers including tunica mucosa, tunica sub-mucosa, tunica muscularis, and tunica serosa/adventitia from exterior to interior.[7] The stomach is divided into the cardia, fundus, and pylorus in terms of structural and functional characteristics of the gastric glands.[8]

Somatostatin (SST) assists in ensuring communication between nerve axons found in the sub-mucosa of gastrointestinal system and connective tissue cells. In the stomach, it suppresses gastric acid secretion in parietal cells, chief cells, and G cells[9] as well as gastric evacuation, gallbladder spasm, bile secretion from the liver, bicarbonate secretion from the pancreas, and absorption in the intestinal system. In the gastrointestinal system, SST reduces calcium, glucose, galactose, fructose, glycerol, lactose, amino acid, triglyceride and water absorption rates, and cell proliferation.[9] The SST was used for treatment of diseases like diabetes mellitus, acromegaly, pancreatic tumors, pancreatic cholera, acute variceal and acute ulcer hemorrhages, dumping syndrome, duodenal ulcer, Alzheimer's, and senile dementia.[10,11]

The stomach is the most important organ affected by the foods consumed and can be damaged at varying rates

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depending on the type of food eaten.[12] Nowadays, studies have focused on substances with protective and therapeutic effects for the gastric mucosa. The SST is a substance released from the gastric mucosa having a beneficial impact on the gastric mucosa, and the consequences of foods consumed on SST secretion were reported previously.[13]

In this study, the aim was to histopathologically examine the changes in rats gastric tissue fed with a high fat diet with cholesterol and to immunohistochemically investigate SST secretion in the stomach.

Materials and Methods

This research received permission from Kafkas University Experimental Animals Ethics Committee, Kafkas University, Kars, Türkiye (Decision Number: 26.01. 2018/001). The laboratory stages of the research were completed in Kafkas University Animal Research and Application Center and Kafkas University Histology and Embryology Department, Kafkas University, Kars, Türkiye.

Experimental design. For this research, sixteen 40-day-old Sprague Dawley male rats were obtained from Ataturk University Research and Application Center, Ataturk University, Erzurum, Türkiye. Animals were kept in cages cleaned daily at 25.00 ± 2. 00 ˚C temperature, and 60.00 - 65.00% humidity with 12-hr light and 12-hr dark cycles, and fed *ad libitum*. After one-week acclimation, rats were randomly divided into two groups and housed with four rats in each cage as follows: Rats in control (n = 8) group had no implementation other than normal feeding (Table 1). Rats in High- fat and cholesterol diet (HFCD) group (n = 8) were fed with pellet feed mixed with butter meeting 65.00% of daily energy requirements and 2.00% cholesterol for 10 weeks (Table 1).[14] At the end of 10 weeks, rats were anesthetized by inhalation of 2.40% sevoflurane (Queen borough, Kent, UK) and the animals were euthanized by cervical dislocation following blood sampling from the ventricular heart. Then, abdomens were opened, and gastric tissue samples were harvested and placed in 10.00% formaldehyde fixation solution.

Table 1. Feeding content (%) of feed given to rats.

Feeding contents* (100 g)	Control	HFCD
Cholesterol	-	2.00
Butter	-	30.50
Moisture	12.80	12.80
Raw protein	23.00	23.00
Raw oil	2.80	2.80
Raw cellulose	5.00	5.00
Raw ash	7.10	7.10
Sodium	0.50	0.50

* Feed components: Corn, soybean meal, sunflower seed meal, corn gluten, sugar beet molasses, calcium carbonate, corn bran, sodium chloride, vegetable oil, sodium bicarbonate, dicalcium phosphate, vitamin-mineral.

* Trace elements (per kg): Vitamin A 1.18 mg, manganese sulfate 144.78 mg, ferrous sulfate monohydrate 375.58 mg, zinc oxide 151.53 mg, copper sulfate 33.51 mg, cobalt carbonate 0.41 mg, sodium selenite 1.07 mg, calcium iodate anhydride 2.00 mg.

HFCD: High-fat and cholesterol diet.

Histological procedure. Rats' gastric tissue samples were fixed in 10.00% formaldehyde solution, passed through graded alcohol and xylol series, and blocked-in paraffin. Sections with 5.00-µm thickness were taken from the blocks and stained with Hematoxylin and Eosin and Crossman's triple staining techniques. Samples were examined using trinocular light microscopy with attached camera (Axio 5; Zeiss, Jena, Germany) to investigate the structural changes in the tissues.

Immunohistochemical procedure. The sections with 5.00-µm thickness were taken from paraffin blocks prepared from gastric tissue and the avidin-biotin peroxidase complex technique was applied to investigate the SST immunolocalization.[15] After deparaffinization and dehydration, the sections were left in 3.00% hydrogen peroxide phosphate-buffered saline (PBS) at the pH of 7.40 for 20 min to prevent endogenous peroxidase activity. Sections passed through PBS were left in sodium citrate buffer (pH: 6.00) in a 600 W microwave oven to reveal antigenic receptors. Sections were incubated in blocking solution A Invitrogen-Histostatin Plus Bulk Kit (Invitrogen, Camarillo, USA) for 8 min and SST primary antibody (Abcam, Cambridge, UK) was applied. After the sections were left for 30 min in biotin secondary antibody, blocking solution C (streptavidin peroxidase) was added and they were left for another 30 min. Then, diaminobenzidine chromogen solution was added and 4 - 5 min later the immunoreactivity status was checked under a (BX51; Olympus, Tokyo Japan); after that, the procedure was stopped with PBS solution. Sections were washed with distilled water and Hematoxylin staining was applied for contrast staining. Sections passed through serial absolute alcohol after staining were left in xylene and then, sealed with entellan (Merck, Darmstadt, Germany). With the aim of determining whether immunohistochemical staining was specific or not, negative controls were provided (all processes were performed under the same conditions using PBS instead of primary antibody). Histological and immunohistochemical investigations were carried out through tissue slides analyses under a light microscope (BX53; Olympus) and then, photographs were taken. Semi-quantitative analyses for all groups investigated the degree of SST immunoreactivity at the cellular level. The numerical density values of immune reactive cells in gastric tissue were determined and calculated using a stereology workstation, consisting of a modified light microscope (Leica DM4000B; Leica Instruments, Buffalo Grove, USA) and Microbrightfield Stereo-Investigator software (version 9.0; Microbrightfield, Williston, USA), as described formerly.[16]

Biochemical analysis. At the end of experiment, blood samples taken from the heart under anesthesia were placed in lithium heparin tubes and assessed for serum total cholesterol levels. Serum total cholesterol was identified using a commercial kit (Cell Biolabs Inc., San Diego, USA). All analyses were spectrophotometrically determined with an Epoch plate reader (Bio-Tek Instruments, Highland Park, USA).

Statistical analysis. The SPSS Software (version 20.0; IBM Corp., Armonk, USA) program was used for statistical assessment. The *t*-test was also used to determine differences between groups. A p value less than was considered as statistically significant.

Results

Body weight. When live body weights before the experiment were compared between control and HFCD groups, there was no statistical difference (265.89 versus 269.39; $p > 0.05$). A significant increase was identified in HFCD groups at the end of the experiment (479.25 versus 421.37; $p < 0.05$).

Biochemical findings. In terms of total cholesterol value, HFCD group was significantly different compared to the control group (101.89 versus 69.17; $p < 0.05$).

Histopathological findings. Gastric tissue of the rats in the control group had normal histological structure in the cardiac, fundic, and pyloric regions (Fig. 1). Gastric tissue of the rats in the HFCD group was identified to have degeneration and inflammatory cell infiltration in the cardiac and pyloric regions. The fundic region of the stomach in the HFCD group had tunica mucosa degeneration and inflammatory cell infiltration was also observed (Fig. 2).

Immunohistochemical findings. The differences between the groups regarding numerical density values of anti- SST reactive cells are seen in Table 2. Somatostatin immunoreactivity in the cardiac, fundic, and pyloric regions of rats' gastric tissue in the control group was more intense compared to the HFCD group (Table 2 and Fig. 3). Gastric tissue in the control group had an intense cytoplasmic SST immunoreactivity being observed in parietal and glandular epithelial cells of the cardiac region and in parietal and chief cells of the fundic region. In glandular epithelial cells of the pyloric region, a moderate degree of cytoplasmic SST immunoreactivity was identified. In gastric tissue of the HFCD group, a moderate degree of SST immunoreactivity was observed in parietal and glandular epithelial cells of the cardiac region and in parietal and chief cells of the fundic region. Weak SST immunoreactivity was determined in glandular epithelial cells of the pyloric region. In negative controls, SST immunoreactivity was not observed in the fundic region (Fig. 4).

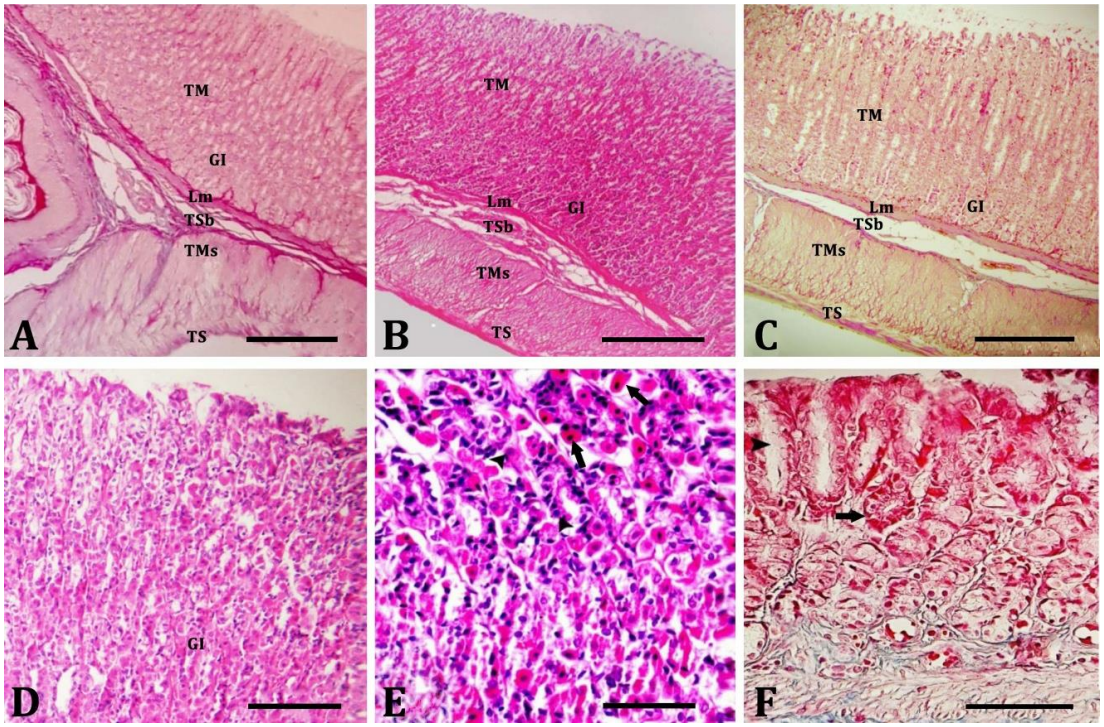

Fig. 1. Rat gastric tissue in the control group. **A** and **D)** Cardia. TM: Tunica mucosa; Lm: Lamina muscularis; TSb: Tunica sub-mucosa; TMs: Tunica muscularis; TS: Tunica serosa; Gl: Glands. **B** and **E)** Fundus. Arrows: Parietal cells; Arrowheads: Chief cells. **C** and **F)** Pylorus. Arrow: Pyloric gland; Arrowhead: Gastric pit, (Hematoxylin and Eosin and Crossman's triple staining; bars = 200 µm in A, B and C; 50.00 µm in D, E and F).

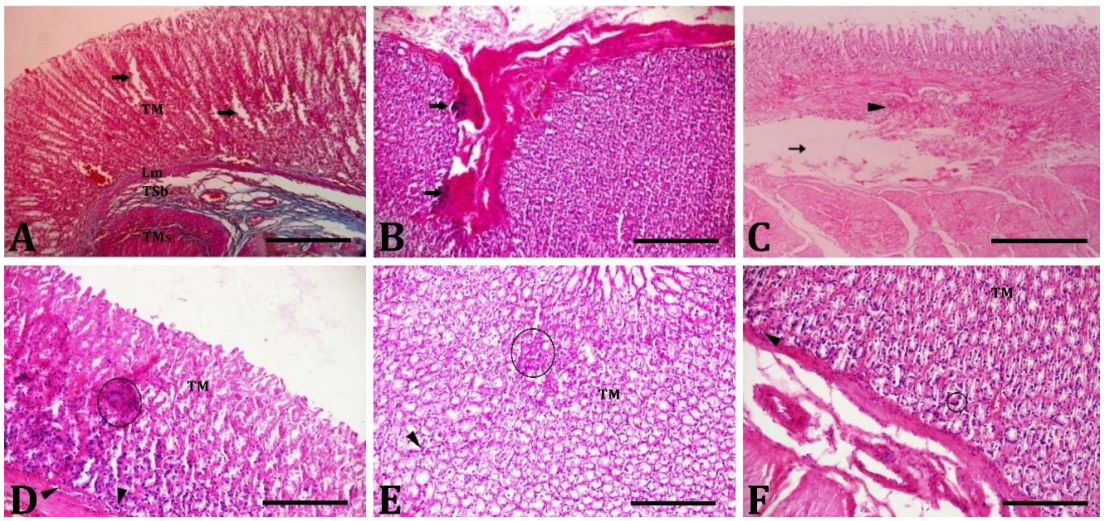

Fig. 2. Rat gastric tissue in the high-fat and cholesterol diet group. TM: Tunica mucosa; Lm: Lamina muscularis; TSb: Tunica sub-mucosa; TMs: Tunica muscularis. **A** and **D)** Cardia. Arrow: Degeneration in lamina muscularis; Arrowhead: Inflammatory cell infiltration. **B** and **E)** Fundus. Arrowhead: Inflammatory cell infiltration; Arrow: Multi-layered smooth epithelium formation; Circle: Degenerated cells in lamina epithelialis mucosae. **C** and **F)** Pylorus: Arrow: Degeneration in lamina muscularis and tunica sub-mucosa; Arrowhead: Inflammatory cell infiltration; Circle: Degenerated cells in lamina epithelialis mucosae, (Hematoxylin and Eosin and Crossman's triple staining; bars = 200 µm in A, B and C; bars = 100 µm in D, E and F).

Table 2. Semi-quantitative analysis of somatostatin immunoreactivity in gastric tissue regions between the groups.

Cells	Cardia		Fundus		Pylorus	
	Control	HFCD	Control	HFCD	Control	HFCD
Parietal cells	+++	++	+++	++		
Chief cells			+++	++		
Glandular epithelium	+++	++			++	+

HFCD: High-fat and cholesterol diet.

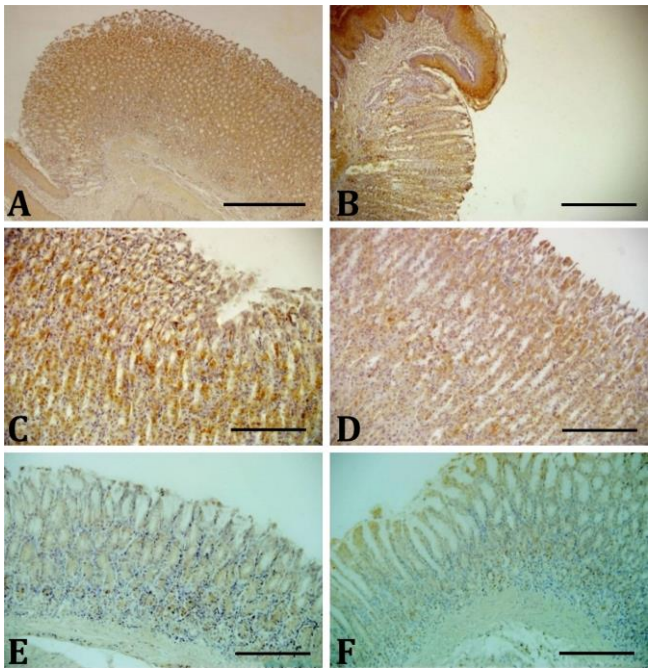

Fig. 3. Somatostatin immunoreactivity of rat gastric tissue. **A)** Control group, cardia; **B)** High-fat and cholesterol diet (HFCD) group, cardia; **C)** Control group, fundus; **D)** HFCD group, fundus; **E)** Control group, pylorus; **F)** HFCD group, pylorus, (Immunohistochemistry staining, bars = 200 µm in A and B; 100 µm in C-F).

Discussion

Nowadays, people consume more foods high in energy, fats, cholesterol, free sugars, and salt/sodium. This type of nutrition causes several metabolic problems in the body.[17] Different studies are available related to the increase in live weight of rats as a result of nutrition with high-fat diets.[18,19] Female Sprague Dawley rats fed with high-fat diet (65.00%) for five months were reported not to have a statistically significant difference between the groups in terms of live weight.[17] The Sprague Dawley rats fed with high-fat diet (45.00% fat rate) for six months had an increase in live weight in four rats; while, there was no increase in live weight for the other four rats.[20] A statistical fall in live weights was reported to be observed as a result of 12 weeks feeding of Sprague Dawley male rats with a high-fat fluid (Liber De Castliq diet).[21] The results obtained in the present research regarding the high-fat diet with cholesterol causing an increase in live weight are in parallel with results from researchers like Bati *et al*.,[19] Cha *et al*.,[22] and Mor and Özcan.[23] The studies having contrary data with our results lead to consideration that results may be linked to animal species, gender, fat proportion in nutrition, stress-induced weight gain cessation in animals or experiments duration.

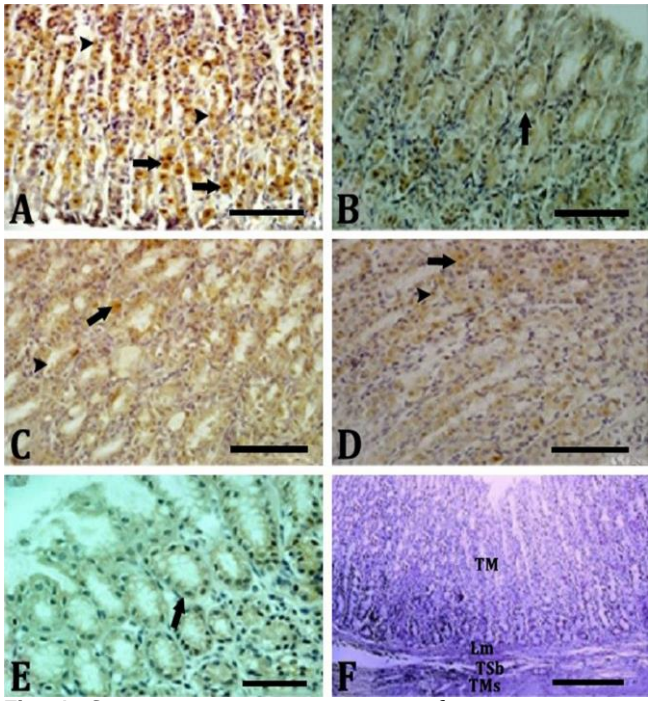

Fig. 4. Somatostatin immunoreactivity of rat gastric tissue. **A)** Control group, fundic region: Arrows: Parietal cells; Arrowheads: Chief cells; **B)** Control group, pyloric region: Arrow: Pyloric gland; **C)** High-fat and cholesterol diet (HFCD) group, cardiac region: Arrow: Parietal cell; Arrowhead: Chief cell; **D)** HFCD group, Fundic region: Arrow: Parietal cell; Arrowhead: Chief cell; **E)** HFCD group, pyloric region: Arrow: Pyloric gland; **F)** Control group, fundic region: Negative control. TM: Tunica mucosa; Lm: Lamina muscularis; TSb: Tunica sub-mucosa; and TMs: Tunica muscularis, (Immunohistochemistry staining, bars = 50.00 µm in A-E; 200 µm in F).

A significant increase was determined in total cholesterol levels of people who were over-weight.[24] Over-weight dogs and rats fed with high-fat diet were reported to have significant serum total cholesterol levels increase.[25] Total cholesterol values were not significantly different for rats fed with 50.00% animal fat diet for six weeks and male Wistar albino rats fed with 45.00 g animal fat for eight weeks; however, those fed for eight weeks were identified to have significant degree of increase in total cholesterol values. [20,25] Increased total cholesterol levels were found in Sprague Dawley rats being fed with 60.00% fat for 12 weeks and 15.00% fat for six weeks.[26,27] Male Sprague Dawley rats fed with high-fat diet containing 1.00% cholesterol, 7.50 % corn oil, and 15.00 % lard were reported to have statistically significant increases in total cholesterol levels.[28] As determined in our research and proposed by several researchers, the increase in total cholesterol level[24,29] is considered to be involved in the occurrence of diseases like cardiovascular diseases and liver steatosis.

As a result of nutrition with a high-fat diet, bile acid reduces in gastric tissue, lesions form in the stomach, parietal cells shrink and wrinkle over time,[30] adipose tissue increases, gastric mucosal structure degrades, and inflammation develops in the stomach causing hypercholesterolemia disease.[31] Rats fed for 12 weeks with a high-fat diet and mice fed for eight weeks generally had hypertrophy, dysplasia, and pseudo-stratification development in gastric tissue, glandular metaplasia, hyperplasia in gastric pits and epithelium, parietal cells morphological changes, and reduced stomach acid. Additionally, parietal cells in the cardiac and pyloric regions found to be degraded and irregular cells took their place, the epithelial integrity of gastric mucosa was degraded, and inflammation was developed.[32] In line with our findings and literature information,[31,33,34] high-fat diet with cholesterol disrupts the gastric tissue integrity and causes increased inflammatory cells infiltration, inflammation, and pseudostratification formation in the gastric tissue, strengthening assumptions that this will increase the risk of gastritis, and gastric ulceration and cancer.

Feeding with a high-fat diet reduces gastric acid secretion from parietal cells and it is reported that gastric acid secretion inhibits SST. [32,35,] During fasting, SST was stated to prevent increased lipid levels by affecting lipid metabolism in liver and adipose tissue of rats.[36] Diabetic rats treated with cinnamon extract were reported to have more intense SST immunoreactivity in mucosal layers, and parietal and chief cells in gastric tissue compared to those in the control and sham groups.[13] Our immunohisto-chemical investigations identified intense SST immuno-reactivity in the fundus as well as parietal and chief cells of the control group, similar to the findings reported by Yıldız *et al*.[13] Furthermore, SST immuno-reactivity was identified in parietal and glandular epithelial cells of the cardiac region and glandular epithelial cells of the pyloric region in the gastric tissue of rats in the control group.

In conclusion, the decrease in SST immune activity and damage to the gastric mucosa were intensely detected in rats fed with a high-fat diet with cholesterol. Based on these findings, SST may have positive effects on the treatment of acute and chronic gastric diseases and prevent complications associated with gastric diseases. In addition, determining the SST immune reactivity of rats gastric tissue fed with a high-fat diet with cholesterol will contribute to the limited literature and more detailed research regarding this subject.

Acknowledgments

The present study was based on Habibe Gündoğdu's Master Thesis.

Conflict of interest

The authors declare that there is no conflict of interest.

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