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Research Article

Immunohistochemical Distribution of Somatostatin in Gastric Tissue of Diabetic Rats Treated with *Cinnamon* Extract [1]

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Abstract

Diabetes is a chronic metabolic disorder, as well as a situation of increased oxidative stress. We examined the distribution of somatostatin in gastric tissues of *cinnamon* extract treated streptozotocin-induced diabetic rats using the immunohistochemistry technique. A total of 30 male *Sprague Dawley* rats were used in the study. The rats were assigned to five groups as control, sham, *cinnamon,* diabetes and diabetes + *cinnamon*. No application was made to the control group, the sham group received intraperitoneally (i.p.) 50 mg/kg sodium citrate, and diabetes was induced by i.p. injection of 50 mg/kg STZ in diabetes and diabetes + *cinnamon* groups. *Cinnamon* extracts were then given to cinnamon and diabetes + *cinnamon* groups by oral gavage at a dose of 200 mg/kg for 14 days. The streptavidin-biotin-peroxidase method was used to determine the immunoreactivity of somatostatin. Gastric tissue sections were prepared and stained by Crossman's triple and Hematoxylin-Eosin staining in order to examine histological structure of the gastric tissue. We determined that somatostatin immunoreactivity of the control, sham and *cinnamon* groups was stronger than for the diabetes, and diabetes + *cinnamon* groups. While a weak immunoreactivity was found in the cardia, fundus and pyloric mucosa of the gastric tissue in the diabetes and diabetes + *cinnamon* groups, a strong immunoreactivity was found in the *cinnamon*, sham, and control groups. Also, a statistically significant was observed when all groups compared in terms of count of parietal and principal cells ($P<0.001$). It was determined that there was a statistically significant difference between diabetes, diabetes + *cinnamon* groups and control, sham, *cinnamon* groups in terms of fasting blood glucose levels ($P<0.05$). In conclusion, somatostatin, which plays an important role in gastroduodenal diseases, was found to be lower in the diabetes and *cinnamon* + diabetes groups.

Keywords: *Cinnamon, Diabetes, Gastric, Immunohistochemistry, Somatostatin*

Tarçın Ekstraktı İle Tedavi Edilen Diyabetik Sıçanların Mide Dokusunda Somatostatinin İmmunohistokimyasal Dağılımı

Öz

Diyabet, kronik metabolik bir bozukluk olduğu gibi aynı zamanda da artmış bir oksidatif stres durumudur. Çalışmamızda immunohistokimyasal teknik kullanarak tarçın uygulanan streptozotosin ile diabet oluşturulan ratların mide dokusundaki somatostatinin salınımını inceledik. Çalışmada 30 adet *Sprague Dawley* cinsi erkek rat kullanıldı. Deney grupları kontrol, sham, tarçın, diyabet ve diyabet + tarçın olarak 5 gruba ayrıldı. Kontrol grubuna herhangi bir uygulama yapılmadı, sham grubuna intraperitoneal (i.p.) olarak 50 mg/kg sodyum sitrate uygulandı. Diyabet ve diyabet + tarçın gruplarına i.p. 50 mg/kg STZ enjeksiyonu yapılarak diabet oluşturuldu. Tarçın ve diyabet + tarçın gruplarına tarçın ekstraktı 200 mg/kg olacak şekilde oral gavaj yolu ile 14 gün verildi. Somatostatinin immunoreaktivitesini belirlemek için streptavidin-biotin-peroxidase metotu uygulandı. Mide dokularının normal histolojik yapısını incelemek için Crossman'ın üçlü boyama yöntemi ve Hematoksilen-Eosin boyaması uygulandı. Kontrol, sham ve tarçın gruplarındaki somotostatin immunoreaktivitesi, diyabet ve diyabet + tarçın gruplarından daha güçlü olduğu tesbit edildi. Diyabet ve diyabet + tarçın gruplarında mide dokusunun kardia, fundus ve pilor mukozasında zayıf immunoreaktivite bulunurken tarçın, sham ve kontrol gruplarında güçlü immunoreaktivite bulundu. Ayrıca tüm gruplar parietal ve prensipal hücre sayıları bakımından karşılaştırıldığında istatiksel olarak anlamlı bulundu ($P<0.001$). Açlık kan glikoz değerleri karşılaştırıldığında diyabet, diyabet+tarçın grupları ile kontrol, sham ve tarçın grupları arasında istatistiksel olarak anlamlı farklılık olduğu belirlendi ($P<0.05$). Sonuç olarak; gastroduodenal hastalıklarda önemli rol oynayan somatostatinin diyabet ve diyabet+ tarçın grubunda daha az olduğu tespit edilmiştir.

Anahtar sözcükler: *Tarçın, Diyabet, Mide, İmmunohistokimya, Somatostatin*

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INTRODUCTION

Diabetes mellitus (DM) is a systemic disease which is characterized by hyperglycemia and causes other disorders in the body, because of insufficient level or lack of insulin production or incomplete usage of insulin [1].

Studies showed that most of the diabetic patients use herbal medicines more than the other supplemental therapies because they believe that herbal medicines are natural, and healthy, whereas in poor quality and with improper use, they can be harmful and cause adverse effects [2-4]. *Cinnamon* has been reported to have positive effects on serum lipids and blood glucose. The active component cinnamaldehyde found in *Cinnamon* expresses its effect on blood glucose can be attributed to it [5]. *Cinnamon* is suggested to reduce high blood glucose levels, repair the damaged β cells and have positive effects on diabetes mellitus [6,7]. Mechanism of action for *cinnamon* was suggested to be increased glycogen storage by acting on glycogen synthesis activity through its polyphenols, and strengthened antioxidant and insulin effects through polyphenol type A; *cinnamon* is thus stated to be benefial in glucose tolerence and treatment of diabetes [8-10].

Being a 14-aminoacid peptide hormone that is secreted from hypothalamus and D-cells of islets of Langerhans of the pancreas; somatostatin is known as the factor inhibiting the secretion of growth hormone from hypothalamus [11]. Somatostatin is an inhibitory peptide with a wide-spectral biological activities [12]. It is included in pancreatic, gastric and intestinal mucosa or gastrointestinal system [12,13] and myenteric neurons. It reduces hepatic biliary, pancreatic and gastric acid secretions and decelerates intestinal passage [12].

The aim of this study was to investigate the effect of *cinnamon* on the immunohistochemical distribution of somatostatin which exists in many areas of the body and whose mechanisms of action differ among organs in the gastric tissue of streptozotocin (STZ)-induced experimental diabetic rats, and the changes caused by diabetes in the gastric structure. This study is based on the view indicating that antioxidant properties and pharmacological effects of *cinnamon* in diabetes mellitus, as well as its protective effects against possible harms of diabetes would lead to alternative ways in fields of medicine and pharmacology.

MATERIAL and METHODS

Animals

Ethical approval of Kafkas University Experimental Animals Local Ethical Committee (No: KAÜ-HADYEK/2017-041) was obtained to conduct the study.

A total of 30 male *Sprague-Dawley* rats were used in the study. The rats were kept at 22±2°C, in standard cages under 12-h light-12-h dark conditions and fed *ad libitum* using standard rodent chow and tap water. The rats were divided into 5 groups including 6 animals in each one: control, sham, *cinnamon*, diabetes and diabetes + *cinnamon* groups. No application was made the control group, the sham group received intraperitoneally (i.p.) 50 mg/kg sodium citrate, diabetes group was administered i.p. 50 mg/kg STZ (50 mL citric acid solved in 40 mL disodium hydrogen phosphate buffer solution, pH 4.5) [14]. Diabetes + *cinnamon* group was administered i.p. 50 mg/kg STZ (50 mL citric acid solved in 40 mL disodium hydrogen phosphate buffer solution, pH 4.5) The animals were considered as diabetic, if their blood glucose values were above 250 mg/dL on the third day after STZ injection [15]. And after then *Cinnamon* extracts were then given to *cinnamon* and diabetes + *cinnamon* groups by oral gavage at a dose of 200 mg/kg for 14 days [6]. At the end of the 14th day, body weights of the rats were measured, they were sacrificed under diethyl ether anesthesia, and gastric tissue samples were obtained subsequently.

Histological Examination

Gastric tissue samples obtained were fixed within 10% formalin solution. Following routine procedures, they were embedded into paraffin blocks, and 5 µm sections were obtained. In order to demonstrate histological structure of gastric tissue, the sections were performed Crossman's Triple Staining and Hematoxylin-Eosin (HE) staining [16] methods and examined under light microscope (Olympus BX51; Olympus Optical Co. Osaka, Japan).

Immunohistochemical Examination

The sections obtained from paraffin blocks after de-paraffinization and rehydration procedures, and incubated in 3% H_20_2 prepared in 0.1 M phosphate buffered saline PBS for 15 min, in order to inhibit endogenous peroxidase activity. Then sections were washed in PBS solution. The samples were exposed to maximum temperature in citrate buffer solution, pH 6.0, in an 800-watt microwave oven for 10 min to release the antigens. Afterwards, they were washed again with PBS. In order to inhibit non-specific bindings, Blocking solution A was dropped (Invitrogen Histostain Plus Broad Spectrum Ref. 85.9943). Somatostatin primary antibody (abcam ab183855, diluted at a rate of 1/500) was administered on the sections for 1 h at room temperature and humidity. Rabbit serum without primer antibody served as the negative control. Following incubation of primary antibodies, streptavidin-biotin method [17] was used, which is one of the indirect methods. For this purpose, Broad Spectrum Antibody (Invitrogen Histostain Plus Broad Spectrum (AEC) Ref. 85.9943), towards the species for which primary antibody was produced, was added on the sections and they were incubated at room temperature for 15 min. Subsequently, HRP streptavidin (Invitrogen Histostain Plus Broad Spectrum Ref. 85.9943) was dropped on the sections and incubated at

room temperature for 15 min. For chromogen incubation, 3,3′-Diaminobenzidine tetrahydrochloride (DAB, Dako Corp) Substrate Solution was added [18]. The sections were immersed into hematoxylin for counterstaining. The slides were examined under light microscope and their images were obtained. Percentage and degree of staining in stained cells were scored by using the semi-quantitative method. Degree of the staining was expressed as 0 (no staining), +1 (weak staining), +2 (moderate staining), and +3 (strong staining) [19,20].

Somatostatin positive cells were counted by 100 square ocular micrometer (eye piece graticule) at 40X magnification under Olympus microscope (BX51). All the obtained data was converted to number of somatostatin positive cells per 1 mm^2 unit area [21,22]. Numerical distribution of somatostatin positive cells were observed in six different sections chosen from ten unit area of parietal and principle cells of each animals.

Statistical Analysis

SPSS (20.0) package software was used to evaluate the data obtained in the study. One Way ANOVA test was performed to determine differences between groups (control, sham, *cinnamon*, diabetes, diabetes + *cinnamon)*. The Duncan test was used to compare the differences between the significant groups.

RESULTS

Blood Glucose Levels

Intra-group and inter-group statistical evaluation of fasting blood glucose levels of rats was carried out and the results obtained were given in the *Table1*. There was no statistically significant difference between the 3rd and 17th days in terms of the mean fasting blood glucoselevels of the rats in the diabetes group. However, it was determined that the diabetes + *cinnamon* group had a statistically significant decrease in the mean fasting blood glucose levels on the 17th day (P<0.05). Control, sham and *cinnamon* groups was not statistically significant difference in terms of fasting blood glucose level between the days 3rd and 17th *(Table 1)*.

Histological Results for the Gastric Tissue

Histologically, normal cardia, fundus and pylorus tissue structures were observed in rats of all groups (control, sham, *cinnamon,* diabetes and diabetes + *cinnamon* groups) *(Fig. 1)*.

Immunohistochemical Results

Somatostatin immunolocalization was determined in similar area in the gastric tissue of rats in control, sham, *cinnamon*, diabetes and diabetes+*cinnamon* groups *(Table 2)*. Strong (+3) somatostatin immunoreactivity was found in the cardia, fundus and pyloric mucosa of control, sham and *cinnamon* groups *(Fig. 2a,b,c)* and weak (+1) immunoreactivity in the diabates and diabetes+*cinnamon* groups *(Fig. 2d,e)*.

A weak (+1) cytoplasmic somatostatin immunoreactivity was found in the parietal and principal cells of fundus in the diabetes *(Fig. 3a)*, and diabetes + *cinnamon* groups while a strong (+3) cytoplasmic somatostatin immunoreactivity in the control *(Fig. 3b)*, sham and *cinnamon* groups. Somatostatin immunoreactivity of parietal and principal cells was statistically significant in the control, sham, *cinnamon,* diabetes and diabetes + *cinnamon* groups (P<0.001). Count of somatostatin positive parietal and principal cells in among groups were summarized in *Table 3* and *Table 4*.

DISCUSSION

In the present study, we evaluated the antioxidant which *cinnamon* on distribution of somatostatin in gastric tissue in streptozotocin diabetic rats. Diabetes is a metabolic problem which is increased by oxidative stress. It is concluded that 14 days of *cinnamon* administration increases somatostatin secretion, which has different roles at different stages of life processes such as cell proliferation,

Table 1. *Statistical evaluation of fasting blood glucose levels of rats according to groups*

Group	Day			F
	1st day	3rd day	17th day	
Control	76.67±2.45 ^{bA}	76±2.76 ^{cA}	80.33±2.21 ^{cA}	0.87
Sham	76.67±1.40 ^{bA}	78.33±1.72 ^{cA}	77.83±2.19 ^{cA}	0.22
Cinnamon	87.83±3.59 ^{aA}	77.33±1.85 ^{cA}	78±1.31 ^{cA}	5.74
Diabetes	84.50±1.33 ^{aB}	373.33±6.31 ^{aA}	363.67±6.83 ^{aA}	913.13
Diabetes + *Cinnamon*	88.83±2.98 ^{aC}	331.16±10.27 ^{bA}	245.16±34.55 ^{bB}	34.6
F	5.32	718.67	68.02	
P	0.00	0.00	0.00	

$^{A, B, C}$ The differences between the mean values indicated by different letters on the same line are statistically significant (P<0.05)
^{a,b,c} differences in the values with different letters in the same column were statistically significant (P<0.05)

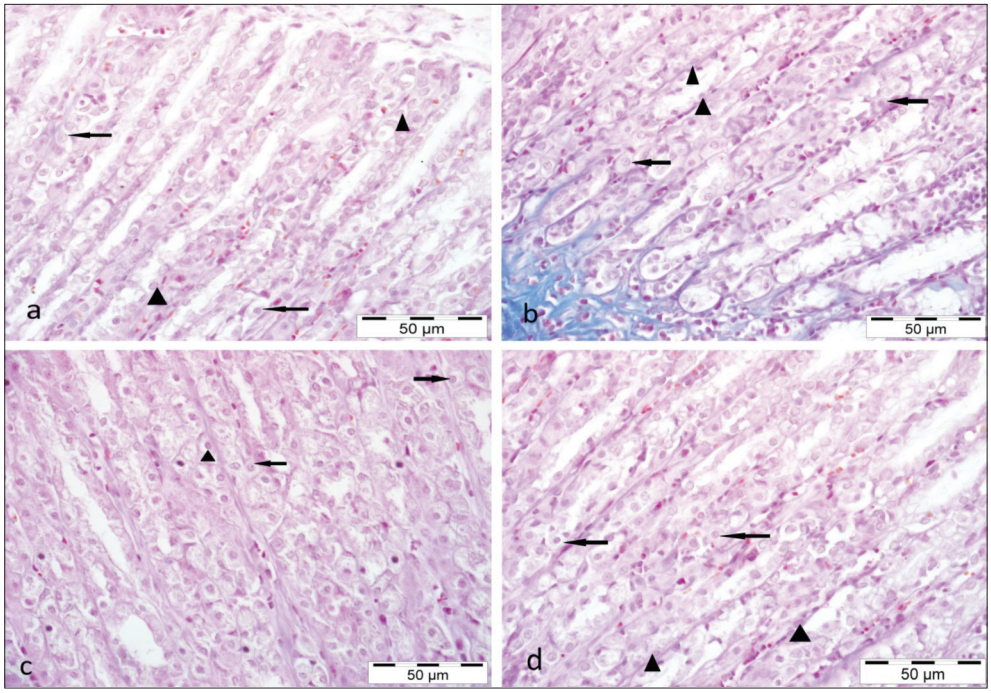

Fig 1. Rat gastric tissue. **a-** Control Group, **b-** *Cinnamon* Group, **c-** Diabetes Group, **d-** Diabetes + *Cinnamon* Group; *arrow:* parietal cells, *arrowhead:* principal cells, Triple, Bar = 50 µm

Table 2. *Comparison of somatostatin immunoreactivity among groups*

Gastric Structures	Diabetes Group	Diabetes + *Cinnamon* Group	*Cinnamon*, Sham and Control Groups
Parietal cells	Weak (+1)	Weak (+1)	Strong (+3)
Principal cells	Weak (+1)	Weak (+1)	Strong (+3)
Pyloric mucosa	Weak (+1)	Weak (+1)	Strong (+3)
Cardia mucosa	Weak (+1)	Weak (+1)	Strong (+3)

cell differentiation, cell migration, tumor growth and apoptosis in rat gastric.

Cinnamon has been shown to lower blood glucose levels, regulate lipid metabolism, suppresses the blood sugar levels by slowing the absorption of carbohydrates from the intestines and have a healing role in type 2 diabetes mellitus with an insulin-like effect [23,24]. Shokri et al.[25] studied three groups 50, 100 and 200 mg/kg doses of *cinnamon* extract daily by gavages for 6 weeks. They determined every doses reduced blood glucose levels. But the dose of 200 mg/kg *cinnamon* extract was the most effective other doses. Kumar et al.[26] in their study investigating the effects of *cinnamon* on blood glucose levels in rats, have administered 150 mg/kg of *cinnamon* extract for 21 days and observed that *cinnamon* had a decreasing effect on blood glucose levels. The decrease in high blood glucose levels and the absence of any toxic effect on the histochemical examination of kidney and pancreatic tissues after a single daily dose of 120 mg/kg *cinnamon* extract in diabetic female and male rats have been considered as positive effects of *cinnamon*. In this case, it was suggested that the *cinnamon* dose is insignificant [27]. In our study, a single dose of 200 mg/kg *cinnamon* extract was administered via oral gavage for 14 days in diabetic rats in the light of literature [6,28]. In our study, the decrease in high blood glucose levels especially in diabetes + *cinnamon* group male rats were similar to some literature studies [6,26-28]. In conclusion, we have determined in our study, which statistically evaluated the effects of *cinnamon* administration on fasting blood glucose that *cinnamon* administration in diabetic male rats may be effective in lowering blood glucose levels.

Diabetes mellitus has been reported to manifest many different pathological situations and damage gastrointestinal system in the long term [29,30]. It has been stated that gastrointestinal symptoms were common in diabetes mellitus which were generally associated with autonomic neuropathy [31]. It was revealed in the study by Bastaki et al.[32] to investigate the morphological alterations in the gastric tissues and parietal cells of streptozotocin-induced experimental diabetic rats with long-term (6 months) that parietal cells were irregularly distributed in diabetic rats compared to those in normal rats, and they reduced acide secretion. The present study revealed no pathological finding in *cinnamon*, diabetes and diabetes + *cinnamon* groups in microscopic evaluation of tissues obtained from STZ-induced diabetic rats. This may be related to the duration of exposure to STZ, which might have changed if STZ was administered to the rats for longer than 14 days.

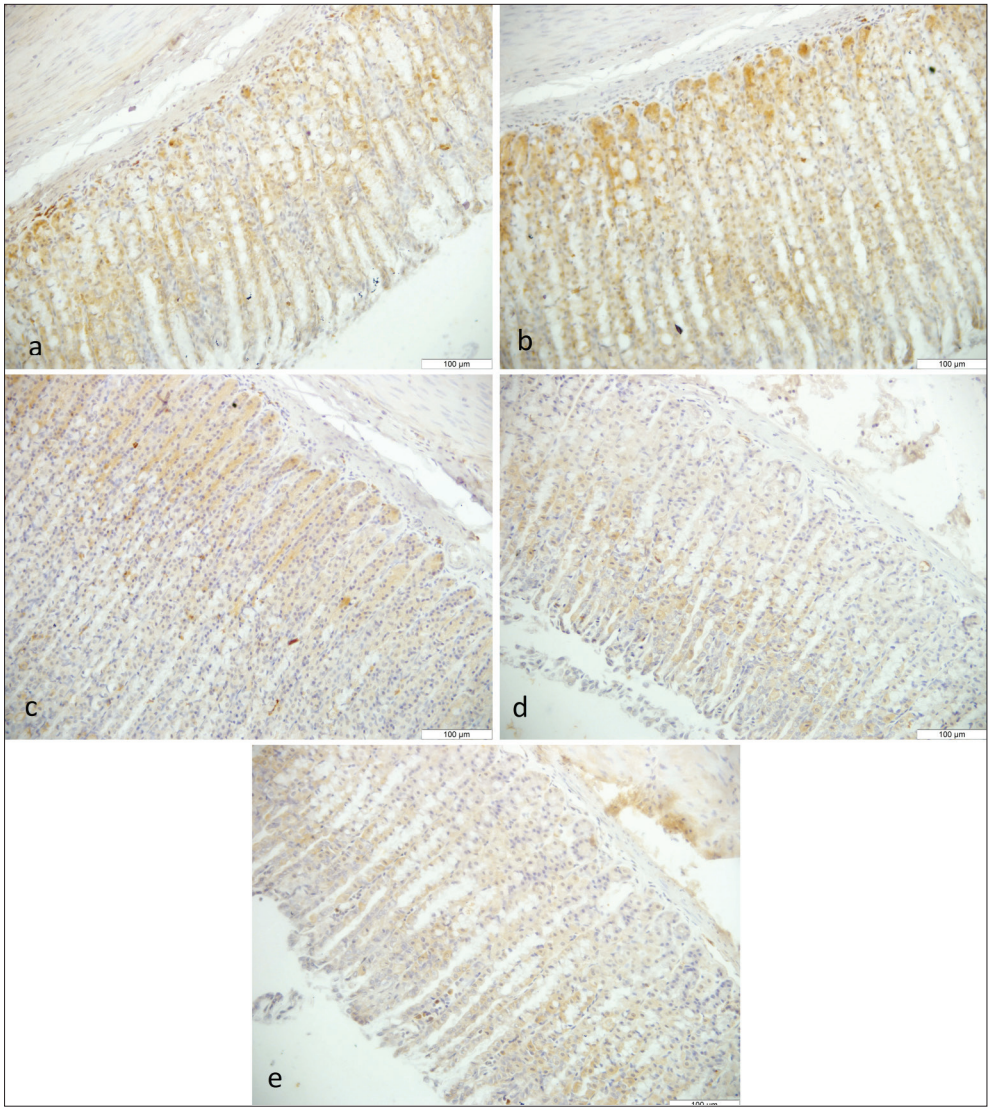

Fig 2. Rat gastric tissue. Intense somatostatin immunoreactivity in control group (a), sham group (b), *cinnamon* group (c); weak somatostatin immunoreactivitiy in diabetes group (d) and diabetes + *cinnamon* group (e). Bar: 100 µm, IHC

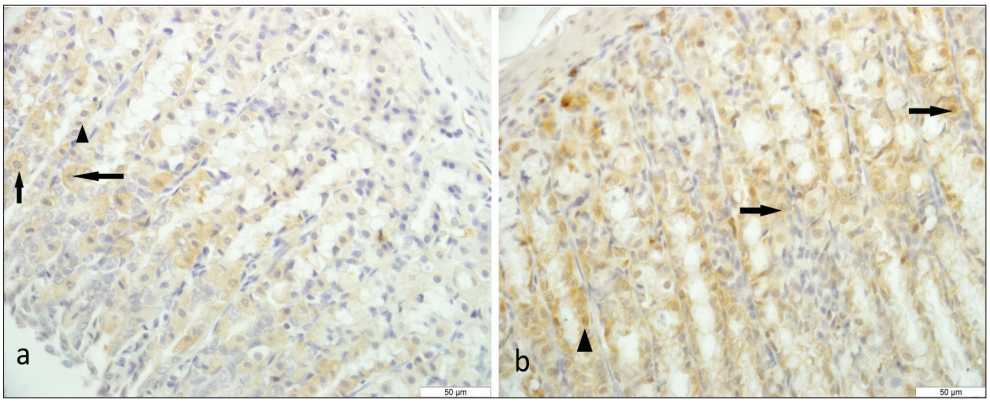

Fig 3. Rat gastric tissue. Weak somatostatin immunoteactivity in principal and parietal cells in diabetes group (a), Intense somatostatin immunoractivity in principal and parietal cells in control group (b), *arrow:* parietal cells, *arrowhead:* principal cells, Bar: 50 µm, IHC

Cinnamon was stated to have an effect to decrease high blood glucose, regulate lipid metabolism, suppress blood glucose by slowing down intestinal absorption of carbohydrates in rats and to likely have a therapeutic role in diabetes mellitus by displaying an insulin-like effect [3,23,24]. Cinnamaldehyde, which is one of the components of *cinnamon*, was determined to reduce blood glucose level in diabetic rats, to increase plasma insulin levels and to regenerate pancreatic β cells damaged by STZ. It has been reported that *cinnamon* releases insulin from β cells and results in a reduction in glucose level, and protects and regenerates β cells via its antioxidant effect [33]. In parallel with the literature review [33-36], somatostatin was determined to display immunolocalization in similar zones in

Table 3. *Comparison of count of somatostatin positive cells in parietal cells among groups*		
Groups	**Number (unit area)**	**M±SD**
Diabetes Group	60	0.96±0.58[a]
Diabetes + *Cinnamon* Group	60	0.80±0.38[a]
Control Group	60	4.10±0.85[b]
Sham Group	60	4.16±0.05[b]
Cinnamon Group	60	4.36±0.67[b]
M: *mean;* ***SD:*** *standard deviation;* [a,b] *Different superscripts in the same column indicate significant differences between groups (P<0.001)*		

Table 4. *Comparison of count of somatostatin positive cells in principal cells among groups*		
Groups	**Number (unit area)**	**M±SD**
Diabetic Group	60	0.96±0.40[a]
Diabetes + *Cinnamon* Group	60	0.70±0.23[a]
Control Group	60	4.10±0.85[b]
Sham Group	60	4.23±0.13[b]
Cinnamon Group	60	4.43±0.68[b]
M: *mean;* ***SD:*** *standard deviation;* [a,b] *Different superscripts in the same column indicate significant differences between groups (P<0.001)*		

control, sham, *cinnamon,* diabetes, and diabetes + *cinnamon* groups in immunohistochemical examinations in the present study. Cytoplasmic and nuclear somatostatin immunoreactivity was observed in parietal and principal cells in fundus area. We determined that on day 14 somatostatin immunoreactivity of the diabetes and diabetes + *cinnamon* groups was weaker than for the control, sham and *cinnamon* groups. Weak immunoreactivity was found in the cardia mucosa and pyloric mucosa of the gastric in the diabates and diabetes+*cinnamon* groups and strong immunoreactivity was found in the control, sham and *cinnamon* groups. It was reported in previous studies that diabetes caused irregular distribution of parietal cells in fundus area [28] In the present study, on the other hand, diabetes was identified to decrease somatostatin immunoreactivity in parietal and principal cells. As a result of these results, the present study revealed that diabetes negatively influenced somatostatin immunoreactivity in fundus area of gastric tissue.

In conclusion, when compared to diabetes groups, *cinnamon* extract administration was determined to increase the secretion of somatostatin which is somatostatin are important regulators of gastric acid secretion. Because we did not found any study on somatostatin immunoreactivity we mentioned in parietal and principal cells, we think that this issue needs to be investigated in more details. This study evaluated whether or not *cinnamon* extract which is reported to be effective in reducing the level of high blood glucose and somatostatin which is reported to be secreted from enteroendocrine cells and to have an inhibiting role on insulin and glucose metabolisms were effective on gastric tissue. We believe that since there is no immunohistochemical study explaining the relationship between somatostatin, *cinnamon,* diabetes and gastric tissue so far, the present study would contribute to literature and further studies should be conducted on the subject.

Declaration of Interest

The authors report no conflicts of interest.

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