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RESEARCH ARTICLE

Anti-Apoptotic and Anti-Inflammatory Effects of Ginger Extract on Small Intestine Tissue in STZ-Induced Diabetic Rats

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ABSTRACT

In this study, it was aimed to investigate the effects of the ginger extract on the TNF-α, IL-1β, bax and bcl-2 expression in the small intestine (duodenum, jejunum and ileum) of rats with experimentally induced diabetes and also, it was examined the ultrastructure of the small intestine by scanning electron microscopy. Wistar albino rats were assigned to five groups as control, sham, ginger, diabetes control, ginger + diabetes. Experimental diabetes was induced by intraperitoneal injection of 50 mg/kg STZ. Ginger and diabetes + ginger group were administered ethanolic ginger extract (200 mg/kg) by oral gavage. In the diabetes control group, it was revealed that intestinal mucosa thickness increased and the villi were folded over each other in the form of a roll and there were disrupted the integrity. Also, while bax, TNF-α, and IL-1β expression increased and bcl-2 expression decreased. In the group treated with ginger, both intestinal histologies and bax, bcl-2, TNF-α, and IL-1β expressions were similar to that of the control group. It was observed that ginger has regulatory effects on inflammatory and apoptotic proteins in the small intestine in diabetes. Ginger can be evaluated in the treatment of diabetes and may provide new targets for therapeutic intervention.

Keywords: Apoptosis, Cytokine, Diabetes, Scanning electron microscopy, Small intestine

INTRODUCTION

Diabetes mellitus (DM) is a multisystemic chronic disease characterised by impaired insulin secretion and hyperglycaemia [1,2]. It was reported that DM causes many morphological and structural damages in gastrointestinal system, such as, gastro-esophageal reflux, intestinal enteropathy, neuropathy involving the gastrointestina [3], diarrhoea, vomiting, habitual constipation and faecal incontinence [4].

Ginger *(Zingiber officinale)* is known to contain more than 400 different compounds. Carbohydrates, lipids, terpenes, and phenolic compounds [5]. It also contains amino acids, protein, phytosterols, vitamins (nicotinic acid and vitamin A and minerals) [6]. Supportive and therapeutic effects are known in many gastrointestinal system disorders such as indigestion, early satiety, bloating, gastritis, ulcer, nausea, vomiting [7,8], irritable intestine syndrome [9], epigastric, pain/burning, dysphagia [10].

Tumour necrosis factor-alpha (TNF-α) is a pro inflammatory cytokine constituting 233 amino acids that secreted by the macrophage and T lymphocytes. TNF-α, in particular, has acts as a regulator of intestinal homeostasis under normal physiological conditions, but has complex regulation affected by the expression of active immune cells and other cytokines, and its dysregulation causes problems. Ultimatly, TNF-α causes inflammation, apoptosis, cytotoxicity and production of Interleukin-1β (IL-1β) [11]. IL-1β is an important mediator of the inflammatory response. It was reported IL-1β and TNF-α were effective correlated with increased complications in diabetes [12,13]. Apoptosis involves either anti-apoptotic (bcl-2) and pro-apoptotic (bax) proteins and the balance between these two groups that serve in the regulation of apoptosis and act to promote or suppress cell death. Increased glucose has been reported to increase the expression of both pro-inflammatory agents and pro-apoptotic proteins by creating a chain effect on cytokines [14,15].

For purpose of this study, the effects of diabetes in the small intestine tissue of STZ-induced diabetic rats and the healing effects of ginger extract administered by oral gavage in the small intestine of diabetic rats were investigated by histological, histomorphometric methods,

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and SEM. Anti-apoptotic and anti-inflammatory effects of ginger were also evaluated by immunohistochemical staining.

Materials and Methods

Ethical Approval

All experiments were approved by Tekirdag Namik Kemal University Ethics Committee for animal experiments (Approval no: 09.11.2022/1153).

Animals

The study was conducted using 40 female, 206±6 g, and 4-months aged Wistar albino rats were performed in Tekirdag Namik Kemal University Experimental Animal Application and Research Center. They were housed standard cages under temperature controlled room (22±2°C) and were maintained on a 12 h light/dark cycle and fed with a standard rat pellet diet and water ad libitum.

Preparation of Ethanolic Ginger Extract

Ginger fresh rhizomes were obtained from a local store and authenticated at department of Botany. Gingers were firstly washed and dried in a dark room. Dried ginger rhizomes were mechanically pulverized in a porcelain mortar. The resulting powder mixture was kept in 95% alcohol for 24 h then the mixture was filtered. This process was repeated 3 times in total. All the prepared mixtures were collected together and alcohol was removed in the low speed evaporator. Dose of 200 mg/kg/bw/day gelatinous extract was dissolved in 2% Tween 80 solution before commencement of the experiment [16].

Induction of Diabetes and Experimental Design

A single dose of 50 mg/kg STZ (St Louis, MO, USA) dissolved in 0.1 M citrate buffer (pH 4.5), was injected intraperitoneally (i.p.) to induce diabetes. After 3 days the application, blood glucose values>250 mg/dL (Accu-Chek Instant glucometer, Roche) were considered an indicator for developing diabetes and rats were included in the diabetes control and ginger + diabetes groups [17]. The rats were divided into 5 groups including 8 animals in each one: Control group (n=8): No application was made (untreated group), sham group (n=8): Tween 80 was given to rats by oral gavage, ginger group (n=8): Fresh ginger extract (prepared daily) was given by oral gavage at the dose of 200 mg/kg, diabetes control group (n=8): This group was administered 50 mg/kg i.p. STZ, ginger + diabetes group (n=8): After diabetes was established, 200 mg/kg ginger extract was administered to this group by oral gavage.

Measurement of Fasting Blood Glucose (BG, mg/dL) and Live Body Weight (BW, g)

For determined of blood glucose levels of all animals, blood glucose were measured initial, 3rd days, and after the experimental period (on days 0, 3, and 33). Live body weights were measured daily in all groups from at the first day of the experiment (0) until the end of the experiment on 33rd days.

Histopathological and Histomorphometric Procedure

At the end of 30 days, rats were fasted for approximately 13 h before sacrificing and were anesthetized with sodium pentobarbital (50 mg/kg, intraperitoneally). Then small intestine tissues of each rat (duodenum, jejunum, and ileum) were gently taken up for histological evaluation. Then the segments of the tissues were fixed in 10% formalin for 48 h. After fixation, the samples were processed for routine histological protocols and embedded in paraffin. The sections taken at 4-5 µm and stained with Crossman's triple staining for histological examination [18]. Villus height, and crypt depth were determined in five villi chosen randomly from six sections taken serially from the duodenum, jejunum, and ileum of each animal [19]. Differences between groups were compared statistically.

Scanning Electron Microscopy (SEM)

The tissues were washed twice in 0.1 M phosphate buffer solution (PBS). Subsequent to the washing process, the tissues fixed on 3% glutaraldehyde for 24 h. And then the tissues dehydrated through a graduated-acetone series (25%, 50%, 70%, and 100%). Two samples from each part of the intestine (inner and vertical surface) were removed and placed on stubs. FEI brand, "Quanta FEG 250" model scanning electron microscope, with technology that does not require vacuum, critical drying or coating with gold, was used. Thus, direct images were taken from identified tissues and then recorded [20].

Immunohistochemical Staining

The streptavidin biotin peroxidase complex (strepABC) method was applied in the small intestine. Sections of 4-5 µm thickness were collected on adhesive slides. The sections were processed in citrate buffer solution (pH: 6.0) for 10 min in a microwave oven at 700 watts. Then, tissues kept on hold in 3% hydrogen peroxide (H_2O_2) for 15 min. The blocking solution A was dripped to prevent the nonspecific binding by IHC Kit. Sections were incubated with bcl-2 primary antibody (ab196495, Abcam, Cambridge, MA 02139-1517 USA, 1/200 dilution), bax primary antibody (ab216494, 1/200 dilution), TNF-α primary antibody (ab220210, TNFA/11721, 1/150 dilution), and IL-1β primary antibody (ab205924, 1/300 dilution) were applied on the sections in a humid environment at the ambient temperature for 1 h. Seconder antibody and after streptavidin was dripped on the sections for 30 min. The 3,3′-Diaminobenzidine tetrahydrochloride (DAB) used as chromogen for 10 min

then Mayer's haematoxylin was used for the background staining. Rabbit serum without primer antibody served as the negative control. Sections were evaluated using research microscope (Olympus BX51, Tokyo, Japan). Evaluation of immunoreactivity of bcl-2, bax, TNF-α, and IL-1β were scored. Immunoreactive cells were categorized as having negative, mild, moderate, and intensive [17,21].

Statistical Analysis

In the study, "G. Power-3.1.9.2" program was used. The sample size was calculated at the 95% confidence level. F tests-ANOVA was used fixed effects, omnibus, one-way and compute of sample size were examined [22].

The change in weight and blood glucose values of rats in all groups was analyzed by two-way repeated measures ANOVA, followed by Bonferroni multiple comparisons test. Paired comparisons between weight and blood glucose values of matched subjects were done with paired t test. Data of villus length, and crypt depth were examined for normality distribution and variance homogeneity assumptions (Shapiro-wilk test). If normally distributed, a One-way ANOVA test was applied, and the differences between groups were analyzed with the post-hoc Tukey's test. The differences were considered significant at P<0.05, and the means and standard errors were calculated. In the study, nonparametric tests were used as the data did not provide normal assumptions. Therefore, the differences between the groups were analyzed with Kruskal Wallis and Mann Whitney U tests. Additionally, the differences were considered significant at P<0.05, and the median values (minimum-maximum) were calculated, and the table was made by means.

Results

Results of Statistical Power and Sample Size

At the end of the analysis, Number of groups=5, Output: Noncentrality parameter λ = 22.9189624, Critical F = 2.6414652, Numerator df = 4, Denominator df = 35, Actual power = 0.9639538 and the standardized effect size was found to be 0.7569 based on previous studies, and with a theoretical power of 0.95, the minimum sample size was calculated as 40, with 8 in each group *(Fig. 1)*.

Blood Glucose Levels Results (BG, mg/dL)

The statistical results showed that streptozotocin (STZ) administration increased blood glucose (BG) levels, and ginger treatment significantly decreased the BG levels in the ginger + diabetes group. Also, there was no statistically significant difference in BG levels between control, sham, and ginger groups (P>0.05) *(Table 1)*.

Body Weight Results (BW, g)

At the end of the study, a significant decrease in body weight (BW) gain was observed in the diabetes control and ginger + diabetes groups. When the rats in the ginger group were evaluated in terms of the increase in BW from the beginning to the last day of the study, it was found that there was no statistically significant increase *(Table 2)*.

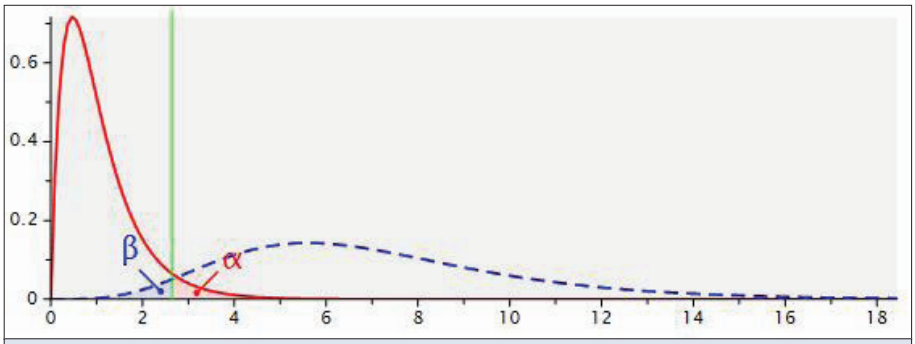

Fig 1. Results of statistical power and sample size

Table 1. *Comparison of blood glucose (mg/dL) change among all groups*

Groups	Initial Blood Glucose (0thDay) (M ± SD)	Diabetic Blood Glucose (3th Day) (M ± SD)	Final Blood Glucose (33th Day) (M ± SD)
Control	78.50±2.29	77.00±2.29	82.50±4.33
Sham	77.75±2.50	80.75±3.00	84.38±3.01[b]
Ginger	88.50±3.40	84.88±1.78	88.00±3.26
Diabetes control	78.25±3.12	270.00±5.05[a]	438.63±21.25[bc]
Ginger + Diabetes	76.75±2.26	261.88±4.46[a]	262.00±22.25[bc]

M: mean, SD: standart deviation. Different superscripts (a,b,c) indicate significant differences between groups. The differences were considered significant at P<0.05

Table 2. *Statistical evaluation of rats body weight changes between all groups (g)*

Groups	Initial Body Weight (0th day) (M ± SD)	Diabetic Body Weight (3th days) (M ± SD)	Final Body Weight (33th days) (M ± SD)
Control	206.00±6.01	229.25±5.07[a]	241.75±5.58[b]
Sham	202.75±4.17	225.50±11.85[a]	243.88±9.85[b]
Ginger	214.50±5.86	232.00±6.48[a]	225.38±6.36
Diabetes control	227.50±2.85	222.25±2.90[a]	170.88±0.72[bc]
Ginger + Diabetes	236.88±9.51	215.25±6.79	209.25±6.82

M: mean, SD: standart deviation. Different superscripts (a,b,c) indicate significant differences between groups. The differences were considered significant at P<0.05

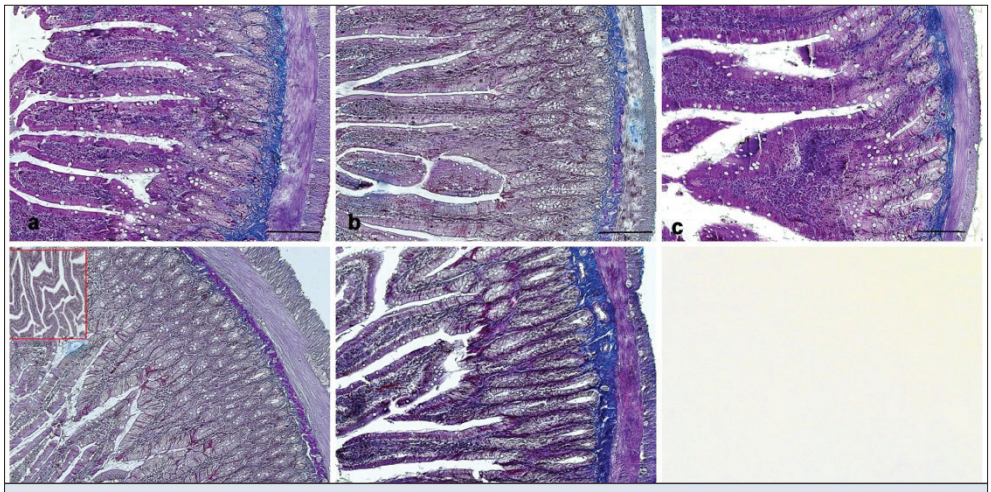

Fig 2. Microphotography of rat duodenum. Control (a), sham group (b), ginger group (c), diabetes control group, longer and irregularly curved villi (small picture) (d), Ginger + diabetes group (e). Crossman's triple staining, bars=200 µm

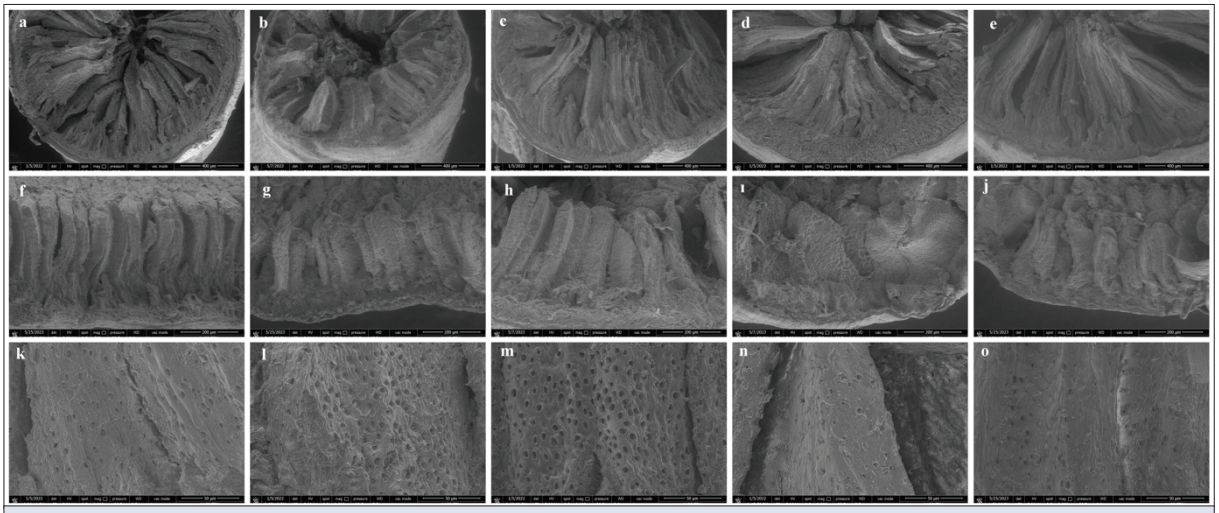

Fig 3. SEM microphotography of rats duodenum, jejunum and ileum. General view of in duodenum of control (a), sham (b), ginger (c), diabetes control (d), ginger + diabetes group (e). Structure of villi in jejunum of control (f), sham (g), ginger (h), diabetes control (i), ginger + diabetes group (j). Goblet cells in ileum of control (k), sham (l), ginger (m), diabetes control (n), ginger + diabetes group (o)

Histopathological and Histomorphometry Result

Normal histological findings were found in the duodenum *(Fig. 2-a,b,c,d,e)*, jejunum and ileum of small intestines of the control, sham and ginger groups. In the diabetes control group, the villi covering the lumen were dense. The Villus height could not be measured in the duodenum due to distortion in the villi *(Fig. 2-d)*. Like as the villus height, crypts depth, the thickness of tunica muscularis and tunica serosa layers increased and this increase was found to be significant in the diabetes control group. *(Table 3)*. In the ginger + diabetes group,

Table 3. Comparison of villus height, crypt depth, thickness of tunica muscularis layer, and thickness of tunica serosa layer among groups

Measurement (µm)	C M+SD	SH M+SD	G M+SD	DC M+SD	G+D M+SD
VH (jejunum)	231.0 (180.6-330.5)	234.7 (134.5-346.8)	229.7 (178.9-307.1)	400.1 [ac] (233.6-578.9)	227.0[b] (166.2-343.8)
VH (ileum)	113.3±1.6	111.2±2.0	108.9±0.9[e]	274.2±7.9[ac]	108.5±1.1 [b]
CD (duodenum)	31.2±0.8	34.0±0.80	33.6±0.8	57.4±1.7 [ac]	35.8±1.1 [b]
CD (jejunum)	25.8 (16.2-41.9)	26.2 (15.2-46.4)	29.30[e] (17.2-40.7)	42.65 [ac] (21.1-88.4)	26.45 [b] (15.1-50.5)
CD (ileum)	20.10 (10.1-34.2)	19.25 (10.1-35.1)	19.65 (10.4-32.4)	36.00[ac] (18.8-79.5)	19.75 [b] (11.2-38.1)
TTM (duodenum)	37.75 (28.6-49.6)	38.95 (35.8-48.2)	40.65 (34.5-43,2)	98.55[ac] (66.1-117.5)	74.70[bd] (54.3-91.7)
TTM (jejunum)	45.10 (34.4-59.5)	36.30 (31.5-53,6)	38.30 (31.5-52.6)	74.90[ac] (55.4-99.1)	61.80[bd] (51.3-81.7)
TTM (ileum)	38.15 (11.4-55.4)	36.45 (20.1-48.4)	30.40 (15.3-43.2)	60.90[ac] (25.5-87.4)	43.30[bd] (26.8-54.2)
TTS (duodenum)	25.00 (17.9-31.2)	25.65 (17.2-33.2)	26.80 (17.8-33.2)	68.50[ac] (49.5-107.1)	50,65[bd] (37.8-57.3)
TTS (jejunum)	24.70 (17.8-31.4)	25.50 (14.60-37.50)	23.45 (16.40-36.20)	63.30[ac] (42.60-98.40)	47.10[bd] (24.30-58.90)
TTS (ileum)	24.65 (19.6-33.8)	26.55 (18.6-30.4)	25.00 (17.6-32.5)	68.10[ac] (48.7-95.8)	37.90[bd] (32.6-48.7)

C: control, SH: sham, G: ginger, DC: diabetes control, G+D: ginger + diabetes. TTM: thickness of tunica muscularis layer, TTS: thickness of tunica serosa layer, VH: villus height, CD: crypt depth, M: mean, SD: standart deviation. a,b,c,d,e Values within a row with different superscripts differ significantly at P<0.05. a: Diabetes group versus control group, b: ginger + diabetes group versus diabetes group, c: diabetes group versus sham group, d: ginger + diabetes group versus ginger group, e: ginger group versus control group

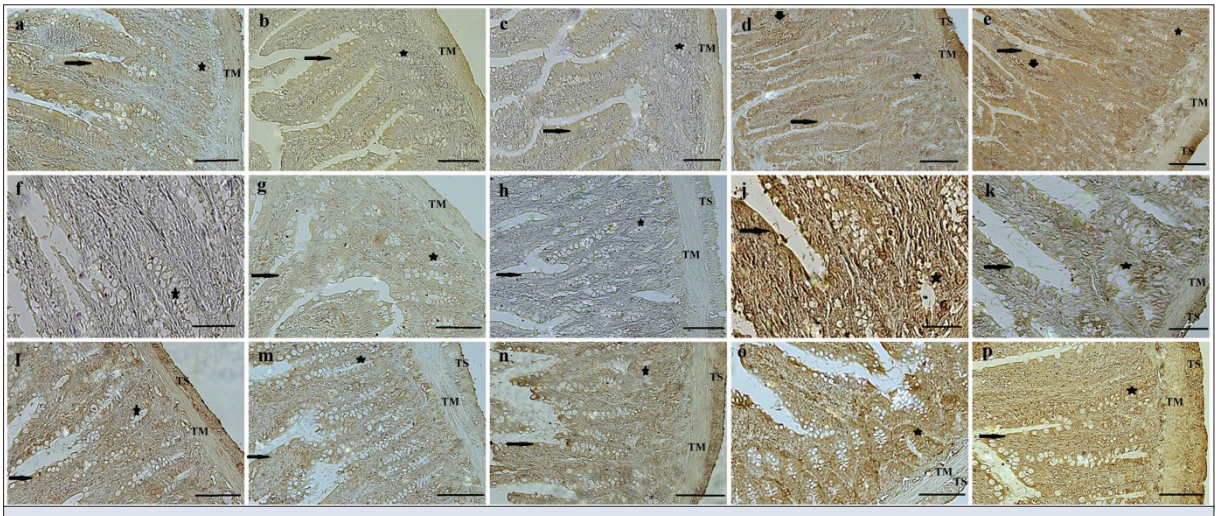

Fig 4. Bax expression in duodenum (a, b, c, d, e), jejunum (f, g, h, j, k) and ileum (l, m, n, o, p) of rats. Control group (a, f, l), sham group (b, g, m), ginger group (c, h, n), diabetes control group (d, j, o), ginger + diabetes group (e, k, p). Villus epithelial cells *(arrow)*, crypt epithelial cells *(star)*, connective tissue cells *(thick arrow)*, tunica muscularis (TM), Tunica serosa (TS). Immunohistochemical staining, bars=200 µm

it was noticed that the villi showed a more regular and distinguishable arrangement with each other. There was also a noticeable reduction in the appearance of the folds seen in the diabetes control group. This group showed similar characteristics to control, sham and ginger group in microscopic examinations *(Fig. 2-e)*.

SEM Results

When the general structures of all groups were compared, the control, sham, ginger and ginger + diabetes groups were found to have normal histological findings *(Fig. 3-a,b,c,e)*. In the diabetes control group, the arrangement of the villi was not regular. In addition, an increase was noted in the tunica mucosa, tunica muscularis and tunica serosa layers *(Fig. 3d)*. It was observed that the villi of the duodenum were leaf-shaped, the ends of the jejunum had a blunt finger appearance and the ileum had a tongue-shaped appearance *(Fig. 3, Fig. 4, Fig. 5)*. In the control,

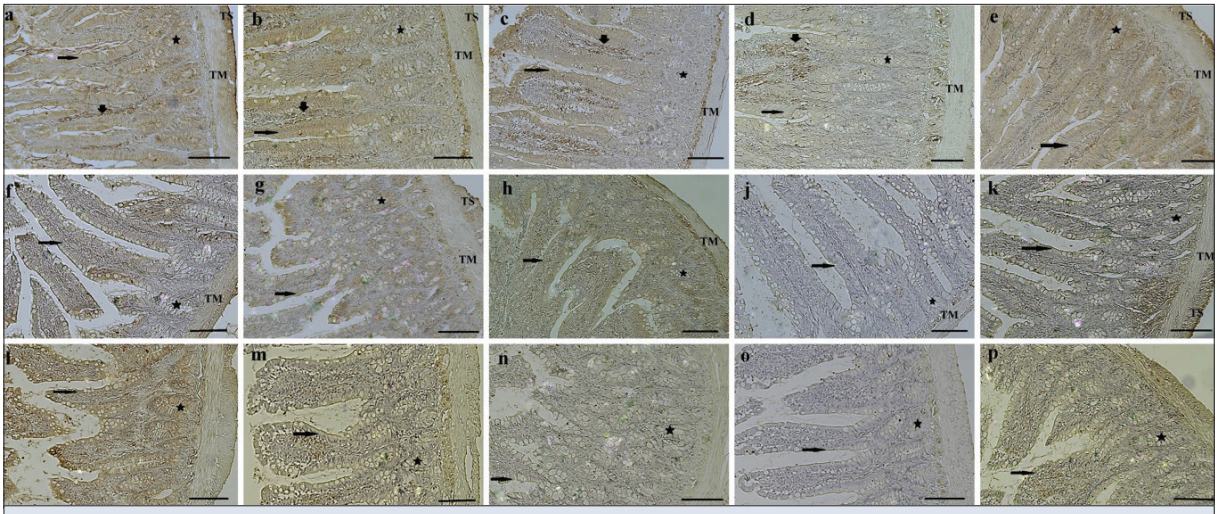

Fig 5. Bcl-2 expression in duodenum (a, b, c, d, e), jejunum (f, g, h, j, k) and ileum (l, m, n, o, p) of rats. Control group (a, f, l), sham group (b, g, m), ginger group (c, h, n), diabetes control group (d, j, o), ginger + diabetes group (e, k, p). Villus epithelial cells *(arrow),* crypt epithelial cells *(star),* connective tissue cells *(thick arrow),* tunica muscularis (TM), Tunica serosa (TS). Immunohistochemical staining, bars=200 µm

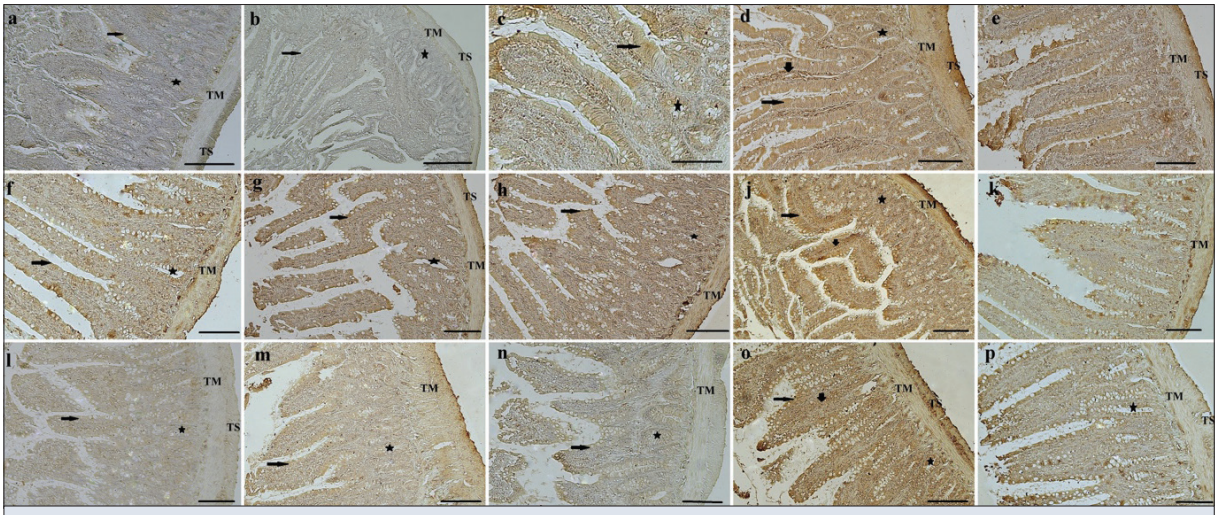

Fig 6. TNF-α expression in duodenum (a, b, c, d, e), jejunum (f, g, h, j, k) and ileum (l, m, n, o, p) of rats. Control group (a, f, l), sham group (b, g, m), ginger group (c, h, n), diabetes control group (d, j, o), ginger + diabetes group (e, k, p). Villus epithelial cells *(arrow),* crypt epithelial cells *(star),* connective tissue cells *(thick arrow),* tunica muscularis (TM), Tunica serosa (TS). Immunohistochemical staining, bars=200 µm

sham, ginger, and ginger +diabetes groups, the villi were arranged parallel and regularly *(Fig. 3-f,g,h,j)*. In the diabetes control group, the villi lay on top of each other and irregular spaces were formed between them *(Fig. 3-i)*.

While the shape of the goblet cells was normal structure in control, sham, ginger group *(Fig. 3-k,l,m,o),* the shape of the goblet cells was contracted and their diameters were decreased in diabetes control group *(Fig. 3-n)*. Ginger + diabetes group was similar to control group *(Fig. 3-o)*.

Immunohistochemical Results

While mild bax expression was observed in villus epithelial cells, crypt epithelial cells, and tunica muscularis and moderate bax expression was observed in tunica serosa of duodenum, jejunum and ileum of control, sham and ginger groups *(Fig. 4-a,b,c,f,g,h,l,m,n)*, intensive expression was detected in the diabetes control group *(Fig. 4-d,j,o)*. In the ginger + diabetes group, moderate intensity expression was detected in villus and crypt epithelial cells, and tunica muscularis *(Fig. 4-e,k,p)*. Also, intensive expression was detected in connective tissue cells *(Fig. 4-e)*.

Moderate bcl-2 expression was observed in villus and crypt epithelial cells, connective tissue cells, tunica serosa and mild expression was determined tunica muscularis in duodenum *(Fig. 5-a,b,c,e)* and mild bcl-2 expression was observed in villus and crypt epithelial cells in jejunum and ileum of control, sham, ginger and ginger + diabetes groups *(Fig. 5-f,g,h,k,l,m,n,p)*. In the diabetes control group, only moderate expression was noted some of connective tissue cells in duodenum *(Fig. 5-d)*. It was not determined bcl-2 expression in jejunum and ileum *(Fig. 5-k,p)*.

Mild TNF-α expression was determined in the duodenum and ileum of control and sham groups *(Fig. 6-a,b,l,m)*, and

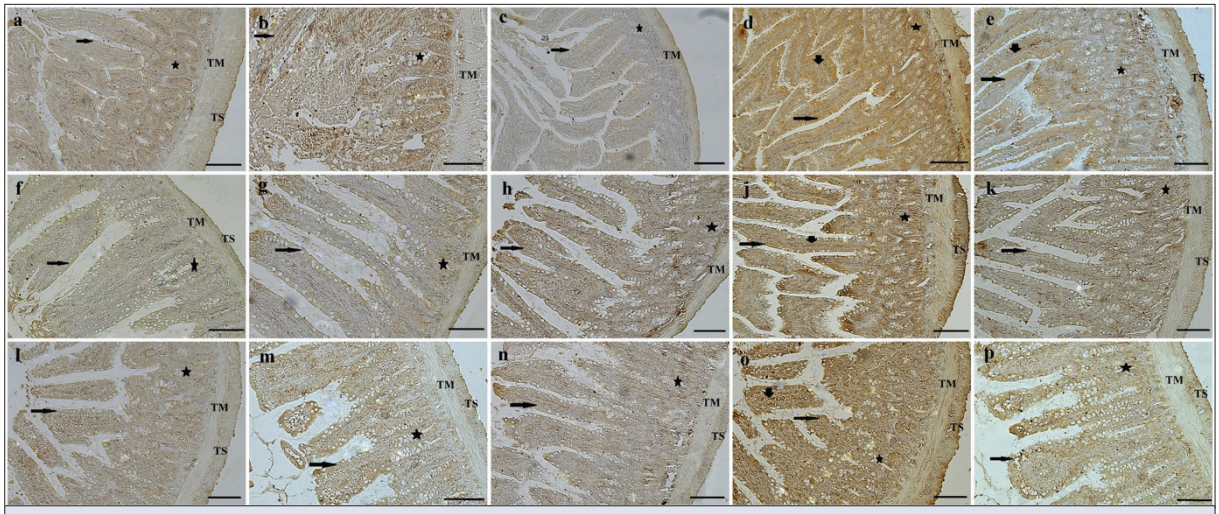

Fig 7. IL-1β expression in duodenum (a, b, c, d, e), jejunum (f, g, h, j, k) and ileum (l, m, n, o, p) of rats. Control group (a, f, l), sham group (b, g, m), ginger group (c, h, n), diabetes control group (d, j, o), ginger + diabetes group (e, k, p). Villus epithelial cells *(arrow),* crypt epithelial cells *(star),* connective tissue cells *(thick arrow),* tunica muscularis (TM), Tunica serosa (TS). Immunohistochemical staining, bars=200 µm

moderate TNF-α expression was noted in in ginger and ginger +diabetes groups *(Fig. 6-c,h,n,e,j,p)* were detected in the villus and crypt epithelial cells and tunica muscularis of the duodenum, jejunum, ileum. Intensive expression was detected in the villus and crypt epithelial cells, tunica muscularis and tunica serosa *(Fig. 6-d,j,o).*

IL-1β expression was mild reaction in the villus and crypt epithelial cells, tunica muscularis, connective tissue cells and tunica serosa layers of all groups except diabetes control group *(Fig. 7-a,b,c,e,f,g,h,k,l,m,n,p).* IL-1β expression was found to be increased in the cells of all layers in the diabetes control group and intensive expression was found in the villus, crypt epithelial cells, connective tissue cells, tunica serosa and moderate expression was found in tunica muscularis *(Fig. 7-d,j,o).*

Discussion

Diabetes is one of the fastest growing diseases worldwide [23]. Many studies reported that ginger extract is responsible for hypoglycaemic activity [16,24,25]. Daily et al.[26] examined several clinical experiments published in 2013-2014 and made a meta-analysis to clarify the evidence for using ginger to decrease blood glucose, and they reported ginger supplementation significantly lowers fasting blood glucose and HbA1c levels. This study was observed that fasting blood glucose increased in the diabetes control group. When it was compared to the diabetes control group with the ginger + diabetic group, it was measured a significant difference between the two groups.

It was reported that body weight decreases in diabetic rats and the loss of weight may be due to excessive degradation of tissue proteins, and protein losing due to unavailability of carbohydrates as an energy source and catabolism of fats. But they indicated that when diabetic rats were fed with ginger for 30 days, their body weight improved. They added that these results may be connected with the fact that ginger contains many bioactive and pharmacological compounds [16]. This study was indicated that a significant decrease in body weight in the diabetes control group and the body weight increased when it was treated with ginger extract for 30 days.

Small intestine morphology can provide information about intestine health. These include hyperplasia and hypertrophy of epithelial cells, increasing crypt depth, villus height and smooth muscle cells [5,27-30]. In studies, light microscopic examination showed that the small intestine tissue of the diabetes group was thicker, the villi and crypts were longer and had deeper volume and surface area compared to the control group [31-33]. Another study was reported that the villus height and crypt depth increased in hyperglycaemia the main reason might be due to a measure taken by the organism against nutrient deficiency and restriction as a result of inadequate nutrition of tissues in the absence of glucose [34]. In this study, it was found that the thickness of tunica mucosa, tunica muscularis, tunica serosa layers, villus height and crypt depth increased in the diabetes control group similar to the previous studies [28-30]. In the group treated with ginger, a regression in the thickness of the mentioned layers was detected. In the study, ginger was found to have an ameliorating effect on the findings detected in the small intestinal mucosa of diabetes.

In a study in which electron microscopic examination was performed, it was reported that the shape of jejunal and ileal villi in diabetic mice was found to be variable; many were twisted, some were conical and others collapsed laterally onto neighbouring villi. Diabetes was reported to increase the adverse effects towards the end of the small

intestine [35]. In the study, it was observed that the villi in the duodenum, jejunum and ileum in the diabetes control group were more elongated, curved and adhered to each other in a wrapped appearance compared to the control group.

One of the main opinion in DM is the view that diabetes causes disruption of intestinal homeostasis, i.e., the balance between cell proliferation and death. Chen et al.[33] found that the expression of TNF-α, IL-1 and IL-6, and bax increased markedly with increasing hyperglycaemia, bcl-2 expression decreased in cell culture. In the present study, the release of bax expression increased while bcl-2 expression decreased in the diabetes group. It has been reported that increased of TNF-α expression activate bax, which cause apoptosis [13]. In the study, increased TNF-α, IL-1β expressions were observed in the diabetes control group. The increase in the expression of bax, one of the pro-apoptotic proteins, together with both cytokines suggests that cytokines have a chain effect with each other and TNF-α has the mentioned inducing effect. In a study was reported that IL-6 levels were higher in diabetics than in non-diabetics. In the 4-year study, they drew attention to the fact that women who did not have diabetes but later developed diabetes had high levels of IL-6 before they developed diabetes. As a result of the study, cytokines may be higher in individuals at risk and inflammatory markers may help early diagnosis of this disease [36].

In conclusion, the study evaluated the effects of diabetes both microscopically and histomorphometrically as well as apoptotic and inflammatory effects. In addition, the ameliorative effects of 30-day 200 mg/kg ginger extract against the adverse effects of diabetes were also investigated. At the end of the study, the villus height, crypt depth, tunica mucosa, tunica muscularis and tunica serosa layers were increased in the diabetes control group. Although diabetes means that organs and/or tissues are deprived of glucose, which is the main source of energy, the fact that it leads to a proliferative result contrary to what is known suggests that there may be different mechanisms. To this end, more extensive studies including food intake, microbiota and hormones are needed. In addition, it was determined that diabetes caused an increase in inflammation, increased TNF-α, IL-1β expression, triggered apoptosis, increased bax release and decreased bcl-2 release. It was observed that ginger extract administered orally decreased and/or eliminated the mentioned negative effects. This study may be a reference for other studies on this subject.

Availability of Data and Materials

The findings of the current study are available from the corresponding author (B. Bakir) upon reasonable request.

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