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The Effects of Tribulus Terrestris on Aquaporin-1 (AQP1) Immunolocalization in Small and Large Intestines of Mice

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Research Article

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ABSTRACT

This study aimed to examine the effects of Tribulus Terrestris on Aquaporin-1 (AQP1) immunolocalization in the small and large intestines of mice. A total of 16 male BALB/c mice were used in the study. The control and experiment groups consisted of randomly selected mice, with each group containing eight animals. Small and large intestine tissues of mice were taken under deep anesthesia at the end of the study. Routine histological and immunohistochemical methods were applied on the intestinal tissues obtained at the end of the study. The analyses indicated that the intestinal tissues of all groups had a normal histology, and there was a statistically significant difference between the numbers of goblet cells of the control and Tribulus Terrestris (TT) groups in the ileum, cecum, colon and rectum. AQP1immunoreactivity was detected in the small and large intestines of both groups. AQP1immunoreactivity increased in the apical cytoplasm of the intestinal villus and crypt epithelium cells in the small intestine of the TT group. Application of TT increased AQP1immunoreactivity and might have a role in absorption activities in the intestines.

Keywords: AQP1, intestine, immunolocalization, Tribulus terrestris

Tribulus Terrestris'in Fare İnce ve Kalın Bağırsaklarında Aquaporin-1 (AQP1) Salınımı Üzerine Etkisi

ÖZ

Çalışmada Demir Dikeni'nin (Tribulus Terrestris) fare ince ve kalın bağırsaklarında Aquaporin-1 (AQP1) immunolokalizasyonu üzerine etkisinin incelenmesi amaçlandı. Çalışma için 16 adet BALB/c cinsi erkek fare kullanıldı. Kontrol ve deney grupları; her bir grupta 8 hayvan olacak şekilde rastgele seçilen farelerden oluşturuldu. Çalışma sonunda alınan bağırsak dokularına rutin histolojik ve immunohistokimyasal yöntemler uygulandı. Yapılan değerlendirmelerde tüm grupların bağırsak dokularının normal histolojide olduğu, kontrol grubu ile demir dikeni (DD) grubu arasında goblet hücresi sayısı bakımından ileum, sekum, kolon ve rektumda istatistiksel olarak anlamlı bir fark olduğu belirlendi. İki grupta da ince ve kalın bağırsaklarda AQP1immunoreaktivitesi belirlendi. DD grubunda villus intestinalisler ve kript epitel hücrelerinin apikal sitoplazmasında AQP1 immunoreaktivitesinin arttığı tespit edildi. Sonuç olarak DD uygulamasının AQP1 immunoreaktivitesini arttırdığı ve buna bağlı olarak bağırsaklarda meydana gelen sindirim emilim olaylarında rolleri olabileceği düşünüldü.

Süreç

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Introduction

Tribulus Terrestris (TT) is a plant that is utilized in folk medicine against cardiovascular diseases, impotence, abdominal distension and edema in certain countries such as India, China, Bulgaria and South Africa (Kostova et al., 2002). The fruit (Caltrop fruit) and roots of this plant contain metabolites with pharmacological significance such as phytosterols, flavonoids, alkaloids and glycosides (Wu et al., 1996). TT is used within traditional medicine as a diuretic or against colic pain, hypertension and hypercholesterolemia (Arcasoy et al., 1998). TT also delays increases in blood glucose levels and inhibits the a-glucosidase activity in the small intestines (Zhang et al., 2006).The small intestine consists of three sections: duodenum, jejunum and ileum. It is the longest and most significant section of the digestive system. Both digestion and absorption take place in the small intestine. The last section of the digestion system consists of the large intestine which has three sections: cecum, colon and rectum. The intestinal mucosa has projections called villi where absorption occurs (Aktümsek, 2020).

Aquaporins (AQPs) are membrane channel proteins from the water channel protein family. They have a small hydrophobic structure facilitating the flow of water (Huang et al., 2006). AQPs are responsible for organizing the water balance. They serve their function by ensuring the constant and rapid permeability of water with a low activation energy throughout the epithelium (Brown et al., 1993; Wintour, 1997). In mammals, there are 13 types of AQPs, ranging from AQP0 to AQP12. Also known as Type 1 AQP, AQP1 weighs 28 kDa and is a membrane protein responsible for channel formation (Preston, 1992). AQP1 plays a role in many important physiological processes. By taking part in fluid emission and re-absorption, it helps ensure water homeostasis and regulate neuro-homeostasis, digestion and body temperature (Sui, 2001). AQP1 is also present in lung, kidney, eye, gall bladder and and red blood cells (Calamita et al., 2005; Higa et al., 2000). Additionally, AQP1 is also synthesized from the submucosa in the gastrointestinal system and the endothelial cells of the lymphatic veins in the lamina propria (Koyama et al., 1999; Nielsen et al., 1993). This study examined the effects of TT on Aquaporin-1 (AQP1) immunolocalization in the small and large intestines of rats.

Material and Method

Material

A total of 16 male Balb-c mice aged 40 days were used in the study. The animals were fed ad libitum in cages that were cleaned daily within an environment at a temperature of 25±2°C and with a humidity of 60-65% in a 12-12-hour light-dark cycle, with each cage containing four mice.

Method

The groups of the study were formed as follows: Control Group (n=8): No procedures were applied on the mice in this group.TT Group (n=8): The mice in this group were administered single doses of pure TT extract, which is sold for commercial purposes, in a form dissolved within distilled water through the 6 mg/kg oral gavage method for seven days. At the end of the study, small and large intestine tissues were collected from the mice under deep anesthesia. The tissues were examined within a 10% formol solution for histological and immunohistochemical examinations. Then, they were blocked in paraffin following the routine histological procedures.

Histological examinations

Crossman's triple staining method (Crossman, 1937) was used on the sections from paraffin blocks to examine the general structure of the small and large intestine tissues, while periodic acid-Schiff (PAS) staining (Yediel Aras et al., 2021) was utilized to determine the goblet cells secreting neutral mucin in the intestines.

Statistical analyses

The Statistical Package for the Social Sciences (SPSS) version 17.0 package program was used to assess the data. Moreover, independent-samples t-test was used to determine the differences between the groups in terms of the numbers of goblet cells.

Immunohistochemical analyses

The streptavidin-biotin peroxidase method was used on the sections on the laminae coated with chrome alum-gelatin. Phosphate-Buffered Saline (PBS) buffer was used for washing throughout the entire procedure (0,1 M, pH: 7,2). The sections were incubated for 15 minutes in 3% H2O2 prepared. The maximum heat setting was applied in a microwave for 10 minutes using the citrate buffer solution. Then, incubation was performed in Large Volume Ultra V Block solution for 10 minutes, and the AQP1 (1/250 dilution) (abcam: ab9566) primary antibody was applied on the sections for an hour at room temperature in a moist environment. Additionally, another incubation session was performed for 30 minutes at room temperature using a Biotinylated Goat Anti B Polyvalent solution and a Streptavidin Peroxidase solution. For the chromogen procedure, a DAB-H2O2 (diaminobenzidine hydrogen peroxide) Substrate Solution was added. The Modified Gill III hematoxylin solution was used for counterstaining. For immunohistochemical analysis, the staining-related properties and densities of the target cells were considered. The assessment was made by two independent observers by assigning values from 0 to 3 for no staining (0), weak staining (1), moderate staining (2) and strong staining (3). The sections prepared for the histological and immunohistochemical analyses were examined and photographed under a light microscope (Olympus BX51; Olympus Optical Co. Osaka, Japan). The Image-j (vI. 50i) software was used to count the goblet cells in the small and large intestine tissues of all groups. The number of goblet cells was measured from six fields in eight different sections within a group.

Results

Histological results

The mucosa, muscularis and serosa layers within the small and large intestine tissues of all groups had a normal histological structure (Figure 1, Figure 2), and the goblet cells covering the surface of the villus intestinalis and crypts displayed a PAS-positive reaction (Figure 3, Figure 4).

Statistical results

No statistically significant difference was determined between the control and TT groups in terms of the goblet cell counts in the duodenum and the jejunum ($p>0.001$), while statistically significant differences regarding the counts in the ileum, cecum, colon and rectum were determined ($p<0.001$) (Table 1).

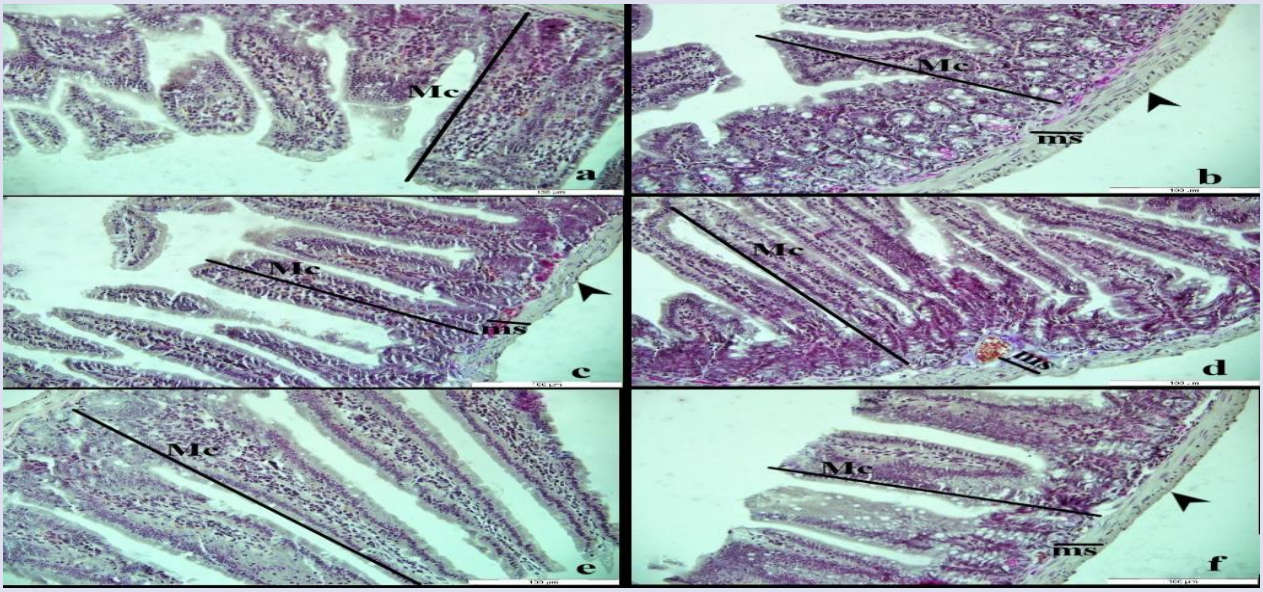

Figure 1. Control and TT group small intestine tissue. a: Control group duodenum, c: Control group jejunum, e: Control group ileum. b: TT group duodenum, d: TT group jejunum, f: TT group ileum. Mc: Mucosa, ms: Muscularis, arrow: Serosa. Triple Staining.

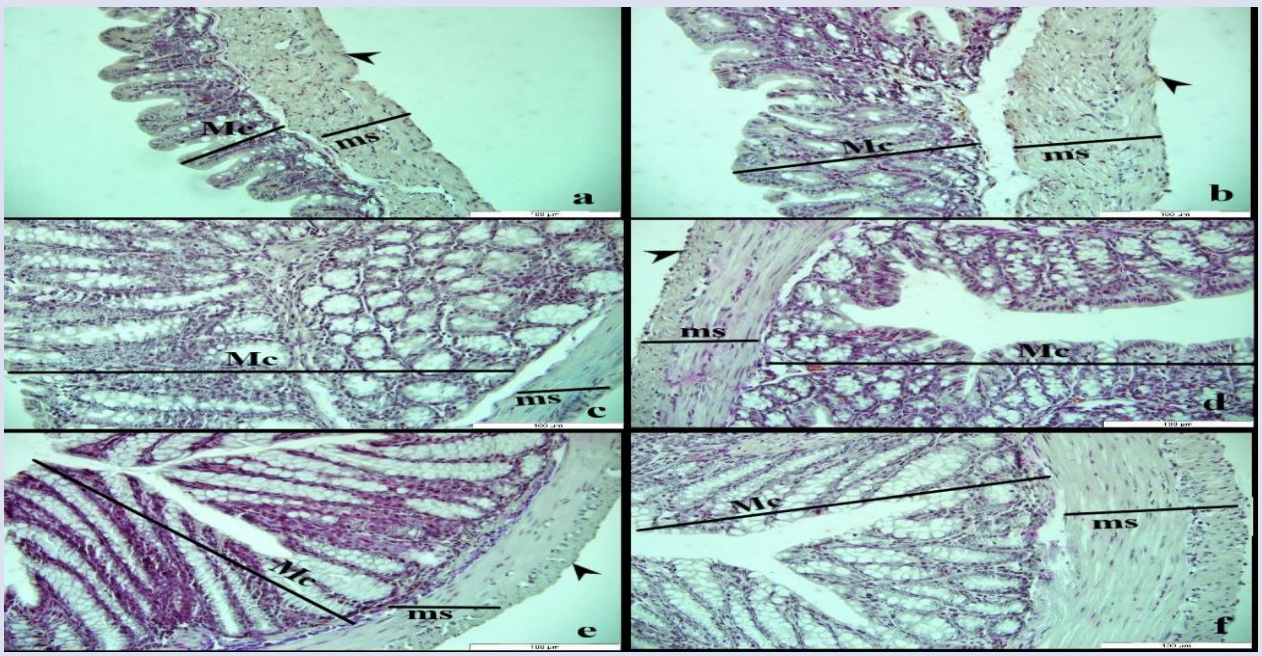

Figure 2. Control and TT group large intestine tissue. a: Control group cecum, c: Control group colon, e: Control group rectum. b: TT group secum, d: TT group colon, f: TT group rectum. Mc: Mucosa, ms: Muscularis, arrow: Serosa. Triple Staining.

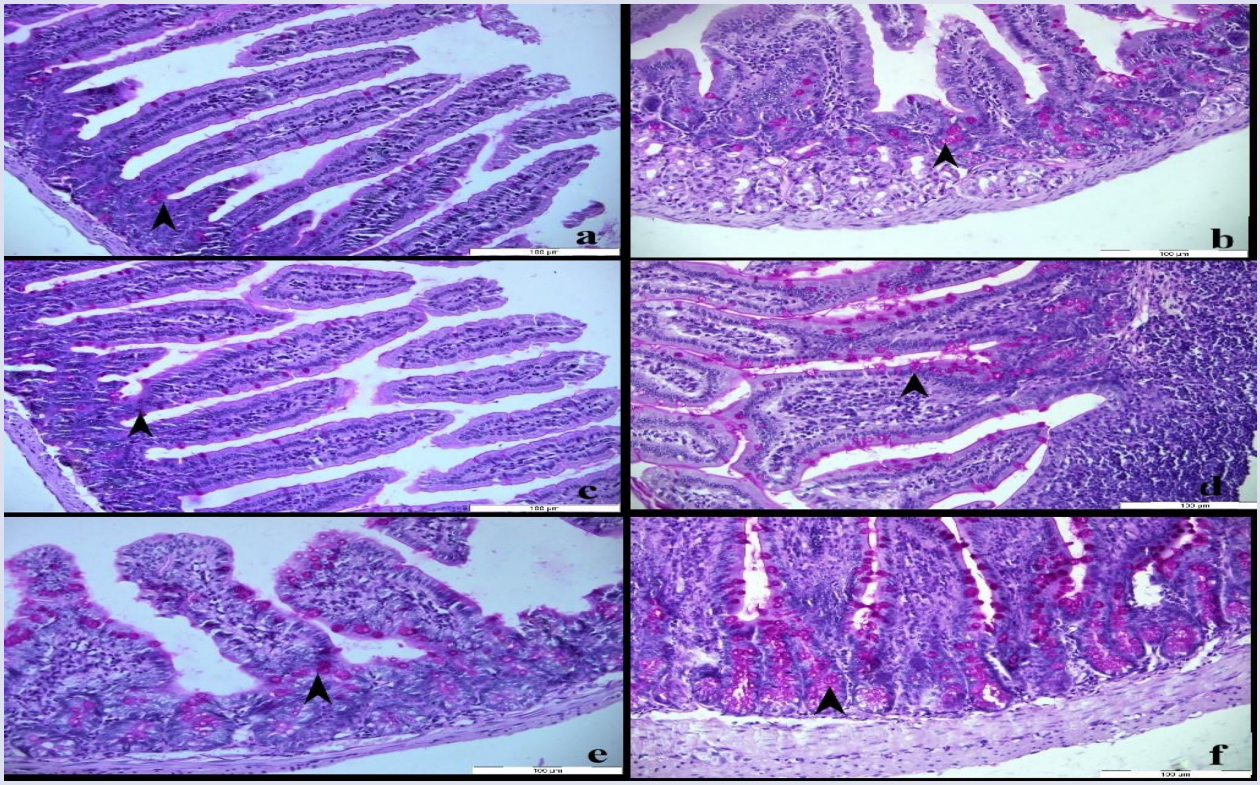

Figure 3. PAS staining in control and TT group small intestine tissue. a: Control group duodenum, c: Control group jejunum, e: Control group ileum. b: TT group duodenum, d: TT group jejunum, f: TT group ileum. Arrow: Goblet cell.

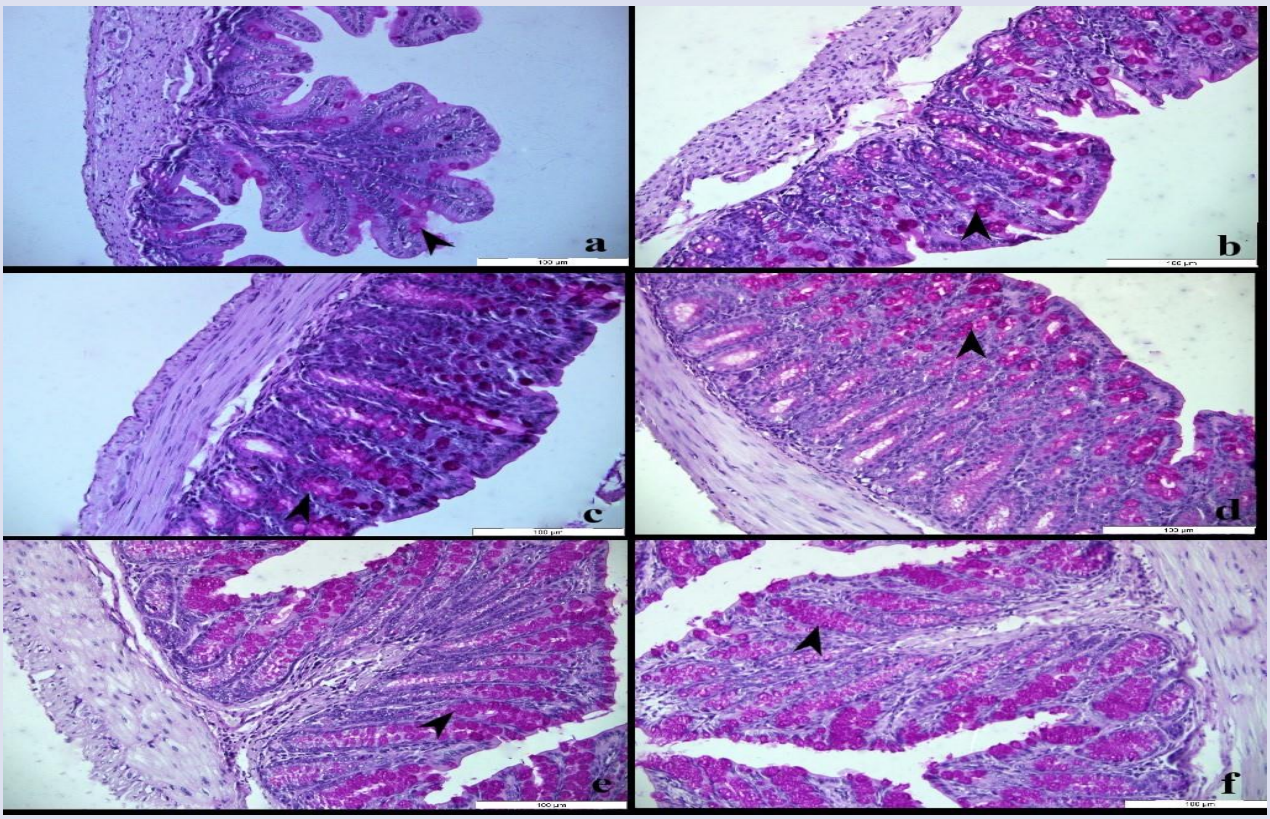

Figure 4. PAS staining in control and TT group large intestine tissue. a: Control group cecum, c: Control group colon, e: Control group rectum. b: TT group secum, d: TT group colon, f: TT group rectum. Arrow: Goblet cell.

Table 1. Statistical evaluation of goblet cell numbers.

Region	Groups	Area	Goblet Cell/100 (µm)	P
Duodenum	Control group	6	46.66±2.10[a]	.006
	TT group	6	60.66±3.40[a]	
Jejunum	Control group	6	48.33±3.80[b]	.004
	TT group	6	64,33±2.07[b]	
İleum	Control group	6	62.5±2.27[a]	.000
	TT group	6	78.33±1.74[b]	
Cecum	Control group	6	66.33±2.88[a]	.000
	TT group	6	91.66±1.11[b]	
Colon	Control group	6	77.5±2.43[a]	.000
	TT group	6	103.33±1.33[b]	
Rectum	Control group	6	107.66±1.83[a]	.000
	TT group	6	129.33±1.68[b]	

AQP1 immunoreactivity in small intestines

Weak AQP1immunoreactivity was found in the apical cytoplasm and serosa layer of the villus intestinalis and crypt epithelium cells within the small intestines of the mice in the control group, while strong AQP1immunoreactivity was present in the vascular endothelium, erythrocytes and connective tissues. In the TT group, weak AQP1 immunoreactivity was found in the serosa layer, while weak immunoreactivity was present in the apical cytoplasm of the villus intestinalis and crypt epithelium cells, and strong immunoreactivity was seen in the vascular endothelium, erythrocytes and connective tissues (Table 2, Figure 5).

AQP1 immunoreactivity in large intestines

Weak AQP1immunoreactivity was seen in the serosa layer of the large intestines of the mice in the control group, while strong immunoreactivity was present in the vascular endothelium and erythrocytes. Weak AQP1immunoreactivity was present in the apical cytoplasm and serosa layer of the villus intestinalis and crypt epithelial cells in the TT group, while strong immunoreactivity was found in the connective tissues, vascular endothelium and erythrocytes (Table 3, Figure 6).

Table 2. The effects of *Tribulus terrestris* on AQP1 immunoreactivity in small intestines tissue.

Area	Groups	
	Control	TT
Intestinal Villus	1	2
Crypt Epithelium Cells	1	2
Serosa Layer	1	1
Vascular Endothelium	3	3
Erythrocytes	3	3
Connective Tissues	3	3

Table 3. The effects of Tribulus terrestris on AQP1 immunoreactivity in large intestines tissue.

Area	Groups	
	Control	TT
Intestinal Villus	0	1
Crypt Epithelium Cells	0	1
Serosa Layer	1	1
Vascular Endothelium	3	3
Erythrocytes	3	3
Connective Tissues	3	3

Discussion

The digestive system is particularly important as it represents the channel where the solid and fluid foods within the organism are separated into small constituents and where the water, vitamins, minerals, carbohydrates, proteins and fats within these constituents are absorbed throughout the digestive channel and transferred into the blood flow (Akbulut et al., 2008). Goblet cells which are present within the intestines in the digestive system perform secretion upon a stimulation. The number of goblet cells increase in sections closer to the colon and rectum (Specian and Oliver, 1991). These goblet cells secrete MUC2-containing granules on the intestinal lumen, while the granules hydrated here constitute the basis of the mucus layer covering the epithelium layer (Van Klinken et al., 1999). This mucus layer helps the intestine surface get fatter and limits the transition of the molecules obtained from the lumen to the mucosa. Similarly, it constitutes a defense line against enteric pathogens (Deplancke and Gaskins, 2001; Specian and Oliver, 1991). After being formed in crypts, goblet cells mature and settle in villi. Approximately 16% of epithelial rat cells consist of goblet cells. The lengths of villi, the diet followed, microbial flora and environmental factors affect the number and volume of goblet cells (Brown et al., 2006; Gersemann et al., 2009; Karam, 1999; Miller et al., 1981; Yunus et al., 2005;).Moreover, an increase in the number of goblet cells may be related to increases in crypt depth. Based on increases in crypt depth, increases in the number of goblet cells have been deemed as epithelial renewal (Gao et al., 2008).

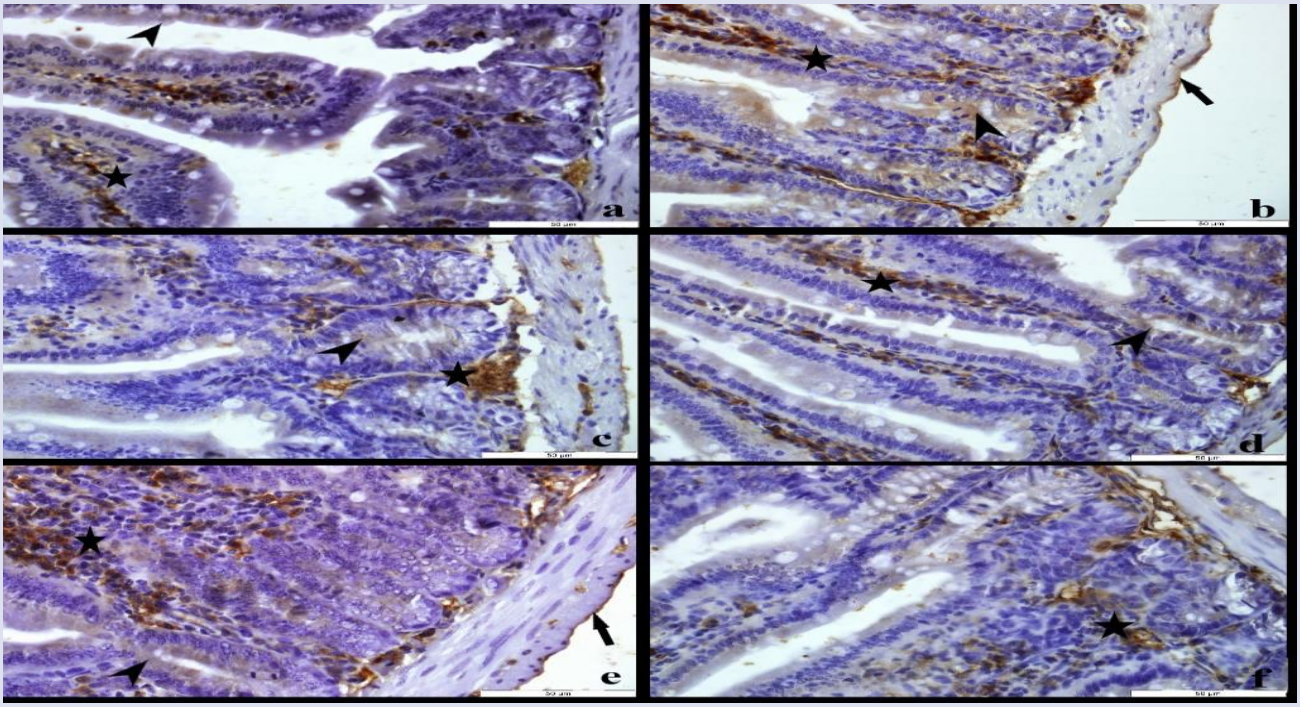

Figure 5. AQP1immunoreactivity in control and TT group small intestine tissue. a: Control group duodenum, c: Control group jejunum, e: Control group ileum. b: TT group duodenum, d: TT group jejunum, f: TT group ileum. Star: Mucosa, arrowhead: Cytoplasm, arrow: Serosa.

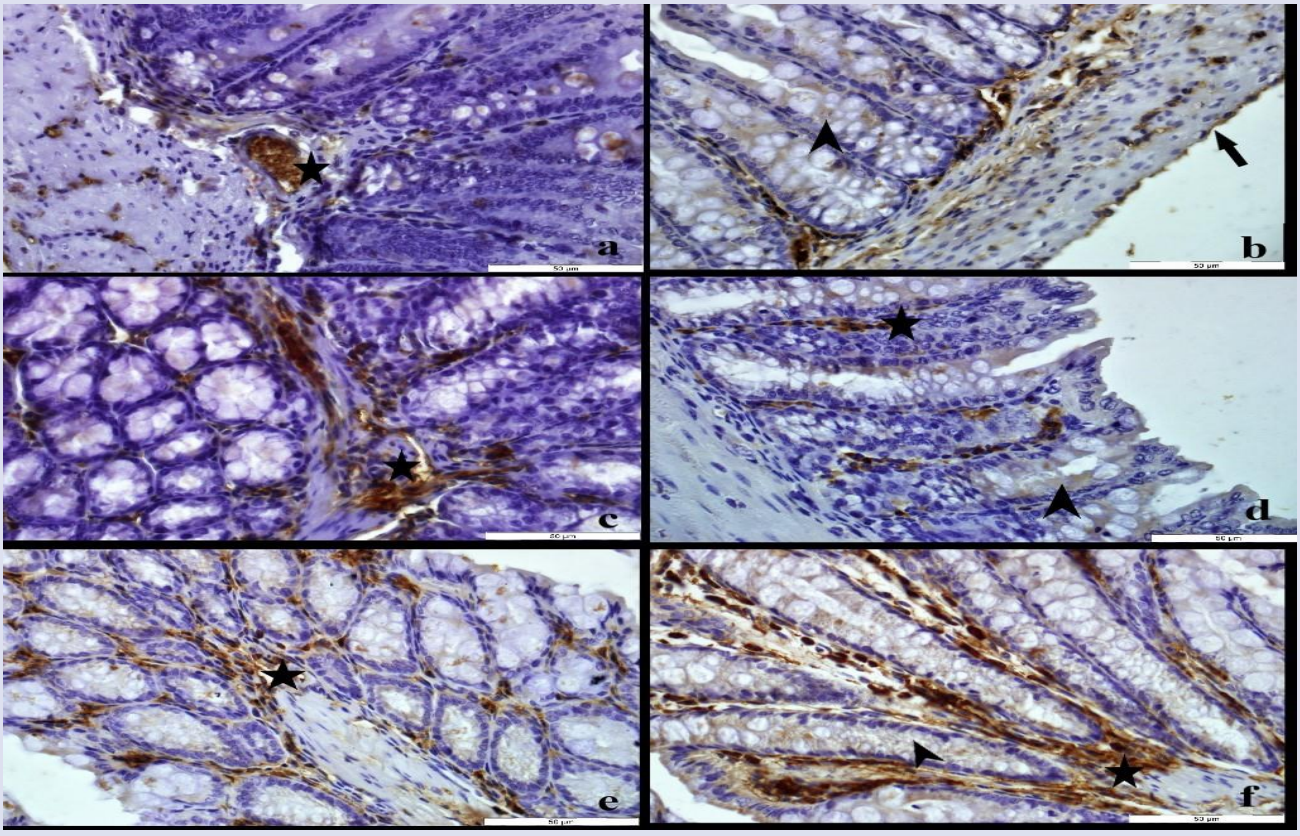

Figure 6. AQP1immunoreactivity in control and TT group large intestine tissue. AQP1immunoreactivity. a: Control group cecum, c: Control group colon, e: Control group rectum. b: TT group cecum, d: TT group colon, f: TT group rectum. Star: Mucosa, arrow: Serosa, arrowhead: Cytoplasm.

However, the number of goblet cells may increase based on damage in the intestinal mucosa. In this case, the activities of goblet cells increase, and the energy and amino acids needed for absorption are used for mucus synthesis. Therefore, increases in the number of goblet cells may adversely affect absorption (Hamedi et al., 2011; Nourmohammadi and Afzali, 2013). Saponins obtained from the fruits of this plant stimulate the non-specific immune response. Moreover, absorption of medicines that are weakly absorbed in the intestines is also boosted (Ayyanna et al. 2012; Tilwari et al., 2011). During the efforts of counting goblet cells within the small and large intestines, a statistically significant difference was present between the groups in terms of their number in the ileum, cecum, colon and rectum. These results suggested that TT application might positively affect an increase in the number of goblet cells within the intestines, particularly the large intestine.

AQP1 plays a role in angiogenesis, wound recovery, organ regeneration and spreading of tumors (Saadoun et al., 2005). Additionally, AQP1 is present in the microvascular structures of the respiratory system and central nervous system, regulating the water permeability of endothelial cells (Verkman, 1998). People with AQP1 deficiency have imperfect urine concentration, while transgenic rats deprived of AQP1 water channels may have imperfect dietary fat process (King et al., 2001; Ma et al., 2001). AQP1 expression in gastrointestinal tissues indicates that the antral (antrum) part of the stomach, oxyntic (the part secreting acid) mucosa and endothelial cells in the gastrointestinal system have low levels of AQP1 expression. Additionally, the gall bladder, as well as the liver and pancreas, were reported to have high endothelial walls, AQP1 expression was not present in the epithelium and mucosa of the small intestine, colon and stomach, and the stromal tissue of the anus had weak levels of AQP1 expression (Mobasheri and Marples, 2004). Small vessels in the villi of the small intestines, enterocytes of crypts and enteric neurons from different animal species were reported to have AQP1 expression (Arciszewski et al., 2011; Cao et al., 2014; De Luca et al., 2015; Matsuzaki et al., 2004). A total of six different AQPs as AQP1, 2, 3, 4, 7 and 8 in the large intestines. However, the distribution of Aquaporin was not always even in the cecum, colon and rectum, and the colon mucosa had high levels of AQP1 expression (Laforenza, 2012; Laforenza et al., 2016). Determining the AQP1 immunoreactivity in the mucosa, serosa and blood vessels of the small and large intestines suggested that AQP1 might have important roles for the digestive metabolism and water transition between the intestinal mucosa and blood circulation. Additionally, the increase in AQP1 immunoreactivity within the apical cytoplasm of the villus intestinalis and crypt epithelial cells within the small intestine cells of the TT group suggested that the TT application might have had positive effects on absorption in the intestinal channels.

Conclusions

Intestines that take a role in digestion and absorption events also constitute the enteric defense line of the body. The intestines perform this function through certain cells they contain. Goblet cells which have a significant place in the physiological processes taking place in the intestines change throughout the intestinal tract, but different factors affect the numbers of goblet cells. The TT plant is utilized in traditional medicine for the treatment of many conditions. This study demonstrated that the numbers of goblet cells significantly differed between the control and TT groups, and the AQP1 immunoreactivity increased in the TT group. Moreover, the results suggested that TT application might have positive effects in preventing any complications within the digestive system and on the intestinal metabolism.

Ethical approval

Ethics committee approval coded KAÜ-HADYEK/2020-10 was received from the Local Ethics Committee for Animal Experiments at Kafkas University on 07.23.2020.

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