

The Effect of Fluoride Toxication on Duodenum, Jejunum and Ileum of Broilers

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ABSTRACT: The aim of this study is to evaluate the effect of fluoride toxication on the contraction and histopathological changes of duodenum, jejunum and ileum of broiler chicken. A total of 80 Ross 308 one-day-old male chicks assigned to 2 treatment groups with 4 replicates of 10 birds per replicated. 1st group was control group and the second group was a fluoride toxicated group, which was given 800 mg / kg flouride with the diet. Between 42 and 49 days of the study, randomly 3 chickens were chosen from each replicate group. Therefore totally 24 chickens were used to determine the contractile activity and examine the histopathological changes in the intestine. The broilers were decapitated and the duodenum, jejunum and ileum preparations were isolated, and mounted under 1 g of basal tension at isolated organ bath. The acetylcholine contractions were examined. There were no significant difference in the contraction of jejunum and ileum preparations, but the duodenum contractions were significantly decreased in fluoride given group as compared to control group, and in fluoride given group 7 of 13 duodenal samples showed no response to ACh. Microscopically; duodenal villus height / villus width ratio was 3.88 in fluoride group, while 6.84 in the control group. This significant ratio difference showed that fluoride could have a toxic effect caused by the villi thickness with severe mononuclear cell infiltration, hyperplasia of the lymphoid follicles and mild haemorrhagia in lamina propria. However there were no histopathological changes in jejunum and ileum between fluoride and control groups. These histopathological results were supported by the pharmacological findings. It is concluded that fluoride toxication affected the duodenal contractions and caused histopathological changes on the normal villus structure of duodenum in the broiler chicken.

Keywords: Fluoride toxication, small intestine, acetylcholine, histopathological changes

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Broyerlerde Florür Toksikasyonunun Duodenum, Jejunum ve Ileum üzerine Etkisi

ÖZ: Bu çalışmanın amacı, broyerlerde duodenum, jejunum, ileumun kasılmaları ve histopatolojik değişiklikleri üzerine flor toksisitesinin etkisini araştırmaktır. Araştırmada kontrol ve deney grubu olmak üzere 1 günlük, 80 adet erkek Ross 308 erkek civciv kullanılmıştır. Araştırma grupları, canlı ağırlıkları eşit ve her birinde 10 adet hayvan olacak şekilde 4 tekrarlı 2 gruba ayrılmıştır. İlk grup kontrol grubu, ikinci grup ise rasyonlarına 800 mg/kg dozda flor katılarak toksikasyon oluşturulan floride verilen gruptur. Çalışmanın 42 ile 49. günleri arası her bir tekrar grubundan rastgele 3 adet tavuk seçilmiştir. Sonuç olarak deneyde toplam 24 adet broyer, bağırsak kasılma aktivitesini ve histopatolojik değişiklikleri belirlemek için dekapite edilmiştir. Ardından duodenum, jejunum, ileum dokuları izole edilip, izole organ banyosunda 1 g ön gerim ile asılarak, asetilkolin (ACh) kasılmaları incelenmiştir. Jejunum ve ileum preparasyonlarının kasılmasında anlamlı bir fark bulunmamıştır. Ancak kontrol grubuna göre duodenum kontraksiyonları flor verilen grupta anlamlı olarak azalmıştır ve flor verilen grupta 13 duodenal örneğinden 7'si ACh'ye yanıt göstermemiştir. Mikroskopik incelemede; duodenal villus yüksekliği / villus genişlik oranı flor grubunda 3.88 iken kontrol grubunda bu oran 6.84 dir. Bu anlamlı oran farkı, florid toksik etkisinin, ciddi mononükleer hücre infiltrasyonu ile villi kalınlaşmasına, lenfoid foliküllerin hiperplazisi ve lamina propriada hafif hemorajiye neden olabileceğini göstermiştir. Ancak, flor ve kontrol grupları arasında jejunum ve ileumda histopatolojik değişiklik olmamıştır. Bu histopatolojik sonuçlar farmakolojik bulguları desteklemektedir. Florür toksisitesinin broyerlerde duodenal kasılmaları etkilediği ve duodenumun normal villus yapısında histopatolojik değişikliklere neden olduğu sonucuna varılmıştır.

Anahtar Kelimeler: Flor toksikasyonu, ince bağırsak, asetilkolin, histopatolojik değişiklikler

INTRODUCTION

Fluorine, an essential element for animals, is widely distributed in nature. However, excess intake of fluoride causes endemic fluorosis (1). Fluorosis refers to fluoride toxicosis especially when it appears in the chronic form (2). Fluoride toxicosis has been well documented in humans and other animals (3, 4). The degree of fluoride toxicity depends on the timing, exposure duration, and dose of fluorine (5). High intake of fluoride for a long duration causes hazardous effects on teeth, bone, and soft tissues (6). Furthermore, fluorosis leads to dental and

skeletal deformation, neurotoxicity, lipid peroxidation, and metabolic disorders (7, 8).

Peristaltic movements, including contraction and relaxation of small intestine, are necessary for the absorption of raw materials, such as protein, carbohydrate, and fat, from food (9). Intestinal mucosal layer is affected by many factors, such as mucosal cell pressure, shear stress, and villus motility. The findings of pathological studies might help understand the effect of these factors and alter the biology of the cells (10).

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Gastrointestinal disorders, such as heartburn, nausea, vomiting, chest and abdominal pain, diarrhea, and constipation, are usually observed with gastrointestinal motility disorders (11). Fluoride absorption occurs in the gastrointestinal tract via passive diffusion without specific transporters (12). Dasarathy et al. (13) showed that the gastrointestinal disorders and mucosal abnormalities are common in patients with osteofluorosis. Luo et al. (14) showed that 800 mg/kg fluoride induces oxidative stress in the intestinal mucosa of broilers. Chronic and acute ingestion of fluoride might lead to both structural and functional alterations in the gastric mucosa (15). Liu et al. (16) showed that 800–1200 mg/kg fluoride in the diet impairs the immune function of cecal tonsil in broilers. Luo et al. (14) reported that 800 mg/kg dietary fluoride inhibits the activity of enzymes superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px), and impaired the antioxidant function in the duodenum, jejunum, and ileum of broilers. The oxidative stress- and inflammatory cell-induced reactive oxygen species might lead to chronic gut inflammation. Decrease in the GSH synthesis have been related to the inflammation of mucosa (17). Inflammation is one of the factors that inhibits intestinal motility (18). Therefore, it is possible that fluoride toxication might affect the contractility of gastrointestinal system.

Although there are many studies on fluoride toxicity, to the best of our knowledge, no study has been conducted on the contraction and histopathological changes in the duodenum, jejunum, and ileum of broiler chicken. In this milieu, the present study aimed to evaluate the effect of fluoride toxicity on the contraction and histopathological changes in the duodenum, jejunum, and ileum of broiler chicken.

MATERIAL and METHODS

Animal husbandry

The experiment carried out at Kırıkkale University Veterinary Faculty Research Poultry Unit, a total of 80 Ross 308 strain, 1 day old male chicks were divided into 2 groups with 4 subgroups which containing 10 chicks in each group. The first group was control group and the second group was fluoride given group. For the control group, the basic diet required for animals was given, and the second group received diet containing 800 mg / kg fluoride. At the end of the 42nd day, 3 animals from each subgroup were selected. Taking this into consideration, a total of 24 broilers were used to evaluate the effect of fluorine broiler on small intestine segments. Kırıkkale University Ethics Committee approved the study protocol (Protocol Number: 2014/41).

Drugs

Sodium fluoride (NaF) was purchased from Merck (Cat. No: 106449). The pharmacological agonist used was acetylcholine chloride (Sigma A6625). The drug were dissolved in distilled water, stock solutions were prepared and kept -20°C. The dilutions were made from stock solution accordingly.

Histopathological and Morphometric Analysis

The birds were necropsied and intestinal tissue samples were collected and fixed in 10% neutral-buffered formalin for 72 h. The tissues were embedded in paraffin wax, sectioned at 4- 5 μ m and stained with hematoxylin and eosin. The prepared slides were examined by light

microscopy (Olympus BX51, Tokyo, Japan) and their photomicrographs were taken (DP25 camera, Japan). The villi height and villi width were selected from the longitudinal sections for each duodenal samples and measured as described by Girgis et al. (20).

Determination of Isolated Duodenum, Jejunum and Ileum Smooth Muscle Responses of ACh in Fluoride Intoxicated Broilers

On the 42nd day of the experiment, the broilers were isolated; afterwards the duodenum, jejunum and ileum smooth muscles were removed. This procedure was continued until the 49th day of the experiment. Until the 49th day the animals continued to feed in the same experimental order. The duodenal segments were isolated from the head of the pancreas lies in the C loop. The jejunum was dissected accordingly. The ileum was dissected between the two ceca of the broiler chickens. The luminal contents were flushed out, and approximately 15 mm long small intestine segments were excised from the middle part. The intestine segments were used as whole tubal preparation, placed in 10 mL tissue bath maintained at 40°C, and filled with Krebs solution (mM: NaCl 118, KCl 4.7, CaCl₂ 2.5, MgSO₄ 1, KH₂PO₄ 1, glucose 11, NaHCO₃ 25; pH: 7.4) (21) and continuously gassed with 95% oxygen and 5% carbon dioxide. The isolated intestine samples were suspended under a basal tension of 1 g, rinsed with Krebs every 15 min, and allowed to equilibrate for 1 h. Only one drug was applied to one suspended ileum of the broiler. Contractions were measured with a force displacement transducer (FDT 05 MAY, Commat, Turkey) and recorded by Biopac System, Inc. USA (MP35). The non-cumulative contraction–response curves were established for ACh (10⁻⁹ to 10⁻³ M) which is a mixed nicotinic-muscarinic agonist.

Statistical analyses

The data obtained from the study was expressed as mean \pm standard errors. The contractions obtained with ACh were calculated as mg contraction. ACh concentrations were compared between itself.

Data were evaluated by the SPSS 15.0 (SPSS. Inc.. Chicago. IL. USA) package. The normality of all data were assessed by Shapiro-Wilk test. According to this, Mann-Whitney U test was used for evaluation of ACh responses on duodenum, jejunum, and student t test for ileum smooth muscle contractions. Differences were considered to be significant when P value was less than 0.05.

RESULTS

The pharmacological results have been presented in Fig 1-3.

The results showed that there was a significantly important difference between the control and fluoride groups at the concentration of 10⁻⁸-10⁻³M ACh. The duodenal contractions were significantly decreased in fluoride given group as compared to control group. At 10⁻⁸, 10⁻⁷, 10⁻⁶ and 10⁻⁴ M ACh concentrations the significance is P<0.05, and at 10⁻⁵ and 10⁻³ M concentration the significance is found as P<0.01. In fluoride given group 7 of 13 duodenal samples showed no response to ACh (Figure 1). No significantly important difference found between the control and fluoride groups at the concentration of 10⁻⁹-10⁻³M ACh on the jejunum and ileum of the broilers (Figure 2, 3).

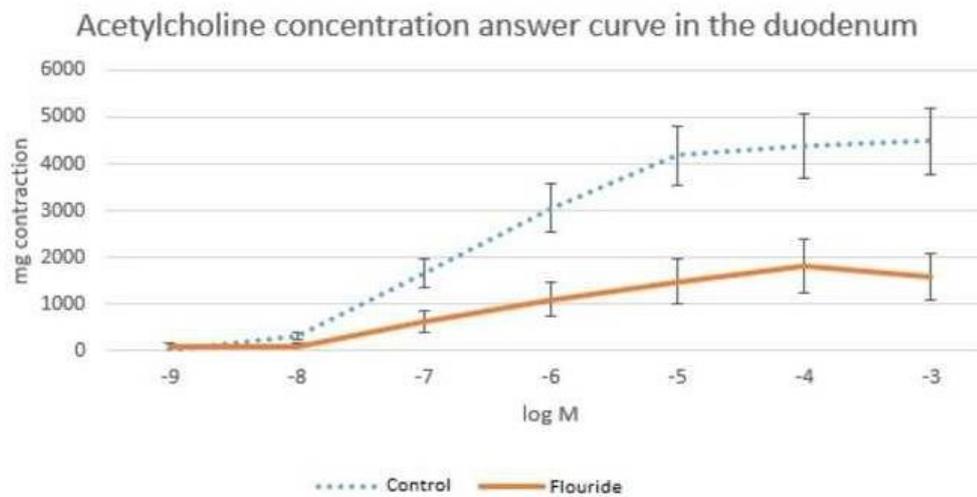


Fig 1. The concentration answer curve of ACh in the isolated duodenum of broilers (there was significantly important difference between the control and flouride groups (* $P < 0.05$, ** $P < 0.01$)). n represents the number of duodenum. control, n:15; flouride, n: 13.

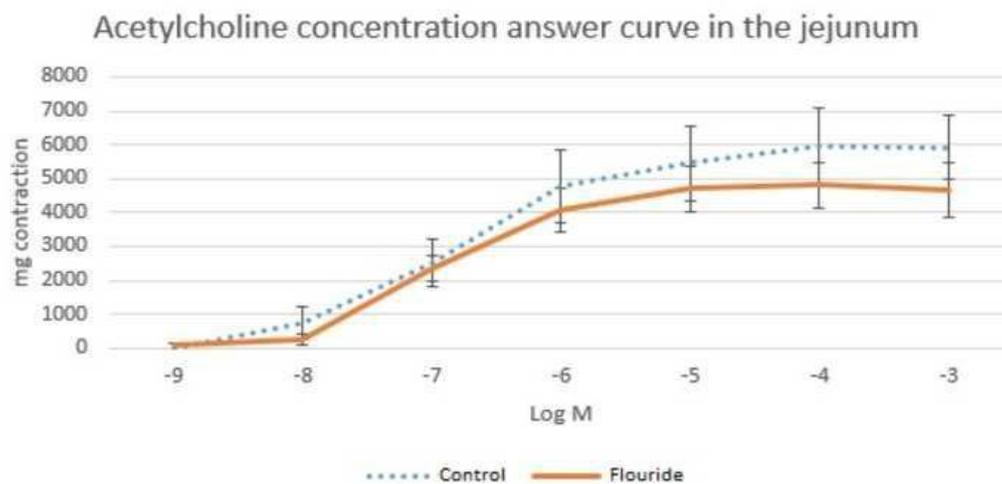


Fig 2. The concentration answer curve of ACh in the isolated jejunum of broilers (no differences were detected between the control and flour groups ($P > 0.05$)); n represents the number of jejunum. Control, n:11; flouride, n:11.

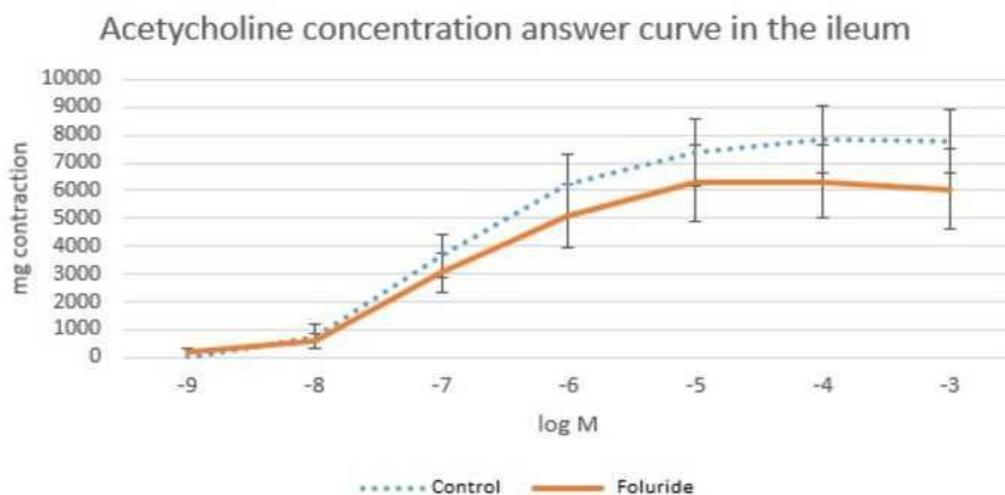


Fig 3. The concentration answer curve of ACh in the isolated ileum of broilers (no differences were detected between the control and flour groups ($P > 0.05$)); n represents the number of ileum. Control, n:11; flouride, n:10.

Histopathologic results

Microscopically; duodenum villus height / villus width ratio were 2.75 / 0.70 in fluoride group, while in the control group these ratio 3.18 / 0.48 (Fig. 4a, 4b and 5).

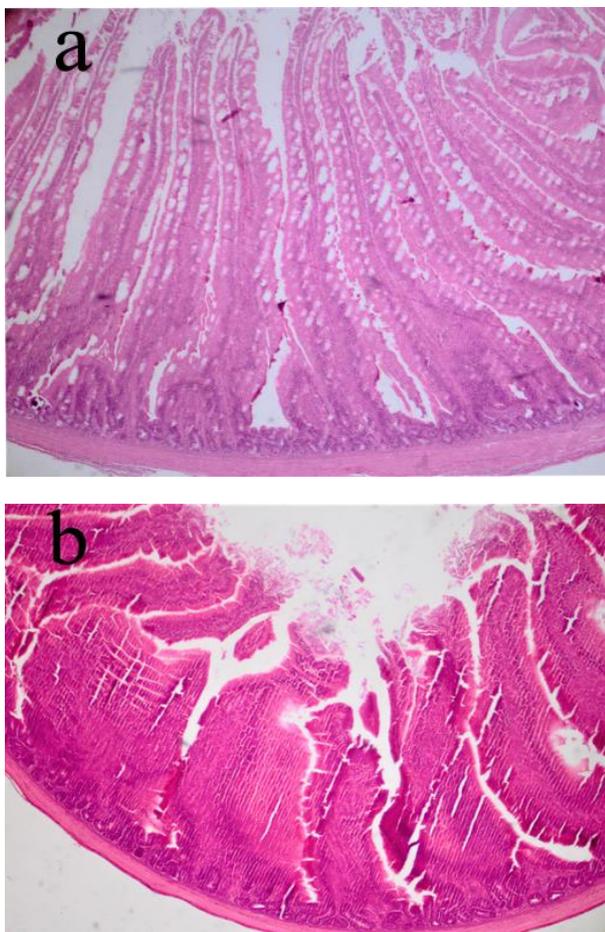


Fig 4. Histopathologic sections of the duodenum of the study groups. Hematoxylin and eosin staining (100x magnification). a. Normal villi in appearance in the control group. b. Increased cellularity in thickened villi and desquamation of villus epithelium in the fluoride group.

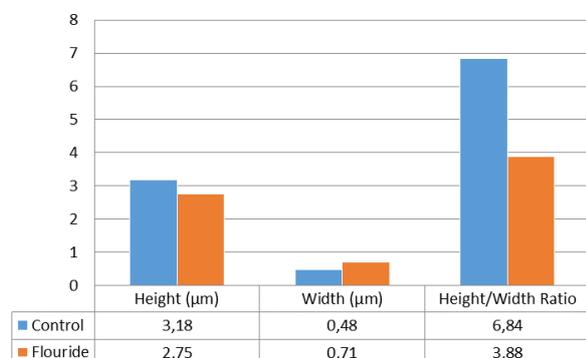


Fig 5. The villi height, width and villus height/ width ratio in the fluoride group compared to control group

The villus height/width ratio of the fluoride-treated group was significantly lower than that of the control group. Furthermore, the desquamation of epithelial cell was observed in the apical region of duodenal villus.

Thickened villi with severe mononuclear cell infiltration and mild hemorrhagia were observed in the lamina propria of the fluoride-treated group compared with that of the control group (Fig. 6). Besides, there was hyperplasia of the lymphoid follicles surrounded by mononuclear cells in the duodenal lamina propria (Fig. 7). Further, there were increased mononuclear cell infiltration in the submucosa and mild inflammation involving macrophages and lymphocytes in the lamina muscularis and serosa.

However there were no significant differences in jejunum and ileum between fluoride and control groups.

DISCUSSION

The stomach is the target organ of fluoride as the gastric mucosa is exposed to the highest fluoride ion concentration (22). The ingested fluoride is absorbed by the stomach and small intestine at a rate of 25% and 75%, respectively in rats (23). Sondhi et al. (24) observed increased number of goblets cells in the villi and crypts, muscular atrophy, cytoplasmic degranulation and vacuolation, and lymphocytic infiltration in the submucosa and lamina propria of swiss albino mice. In a prospective case-controlled study, the patients with osteofluorosis showed gastrointestinal symptoms, such as abdominal pain; and microscopic abnormalities, such as loss of microvilli, cracked clay-like appearance, and presence of surface abrasions on the mucosal cells (13). Shashi (25) showed erosion and necrosis of surface mucosa, hemorrhage, and necrosis in the Brunner gland; and hypertrophy of muscles in the muscularis mucosa of duodenum in sodium fluoride (NaF)-treated rabbits. An electron microscopic study conducted on the duodenal mucosa of rabbits that received NaF revealed a cracked clay-like appearance (26). Das et al. (27), by electron microscopic examination, showed abnormalities in the gastric and duodenal biopsies of human patients who received sodium fluoride. They also observed inflammation in the duodenal mucosa. In the present study, high fluoride intake caused histopathological changes including epithelial desquamation, inflammation, and microvillus height reduction in the duodenum of broiler chicken. Recently, Luo et al. (28) showed that fluoride decreased the length, weight, and viscera index, and suppressed intestinal development in broiler chickens. In the present study, there was a significant decrease in the height/width ratio of fluoride-treated group when compared with that of the control group. Reduced smooth muscle intestinal contractility is thought to be associated with histological and functional disorders, changes in muscarinic receptor activity, differences in the activity of ion channels, and decrease in the activity of the myosin light chain phosphatase inhibitor CPI-17 (29). Inflammation is known to inhibit intestinal motility, and therefore changes the intestinal microflora. The changes in the intestinal microflora play an important role in the pathogenesis of mucosal inflammation, which in turn aggravates intestinal dysmotility. Molecular mechanisms, such as increase in the activity of myosin light-chain phosphatase and alteration in the activity of ion channel in smooth muscle cells (18), are responsible for the motility disorder in the inflamed gut. Further, Vermillion et al. (30) suggested that the patients with Crohn's disease show abnormal smooth muscle contraction in the inflamed small intestine. Fluoride is a known risk factor for inflammatory bowel diseases (31).

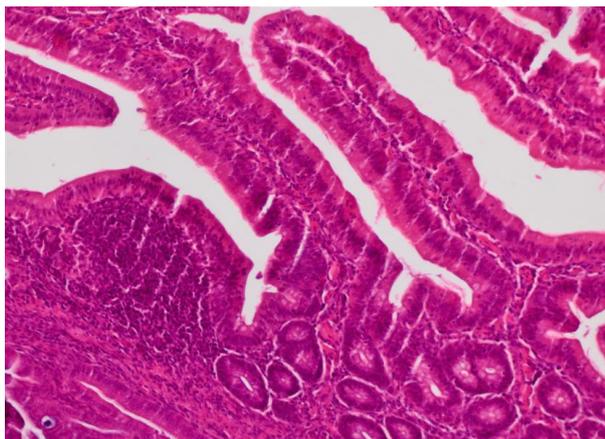


Fig 6. Histopathologic sections of the duodenal Lamina propria of the fluoride group. S Hematoxylin and eosin staining (200x magnification). The infiltrate of lymphocytes and macrophages and haemorrhagia

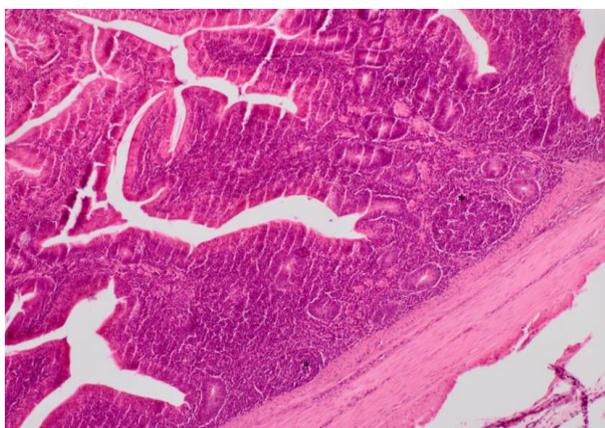


Fig 7. Histopathologic sections of the duodenal lamina propria of fluoride group. Hematoxylin and eosin staining (100x magnification). Hyperplastic lymphoid follicles (asterisk) and mononuclear cell infiltration

In the present study, the contraction response of the duodenum to ACh decreased in the fluoride-treated broilers. Furthermore, in the fluoride-treated group, 7 of 13 duodenal samples showed no response to ACh (Fig 1). Similarly, Sanni et al. (32) showed that *Escherichia coli* infection decreased the duodenal responses to carbachol and histamine in chickens. Koyluoglu et al. (33) have shown that the spontaneous contraction of ileum and jejunum decreases the frequency and amplitude of responses in rats with peritonitis. However, Bassone et al. (34) found a significant increase in the amplitude of contractions in the distal colon of acutely inflamed rats. The altered contraction to ACh in the duodenal segments of broiler chicken observed in the present study can be attributed to the histopathological changes and inflammation of duodenum caused by high fluoride content in the diet. In the present study, ACh contracted the jejunum and ileum of the chicks; however, there was no difference in the contraction between the control and fluoride-treated groups (Figs 2 and 3). Similarly, no histopathological differences were observed in the jejunum and ileum between the control and fluoride-treated groups. Yildirim et al. (35) studied the effect of aflatoxin

supplementation in the diet on the ileum of broiler chickens and found no difference in the contraction of ileum to agonists, such as ACh, bethanechol, and nicotine. Furthermore, similar to the findings of the present study, no histopathological differences were observed in their study.

CONCLUSION

The results of the present study indicate that fluoride toxicity alters duodenal contractions and causes histopathological changes in the normal villus structure of the duodenum of broiler chicken. The pharmacological results agree with the histopathological results. Further studies will help to understand the underlying mechanism of the responsiveness of duodenum to ACh induced by fluoride toxication.

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