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Histopathological Evaluation of Zebrafish (*Danio rerio*) Intestinal Tissue After Imidacloprid Exposure

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Research Article

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Abstract

The use of pesticides has been increasing day by day to increase productivity in agriculture. Pesticides used in the agricultural area mix into the aquatic ecosystem through rains and groundwater etc. and it threatens the life of aquatic organisms. Nowadays, one of the most used pesticide group is neonicotinoids. Imidacloprid is the most well known in this group. In this study, it was aimed to observe the histopathological effects of imidacloprid in zebrafish intestinal tissue. Adult zebrafish individuals were administered imidacloprid in concentrations of 9.5, 19, and 38 $mg.L^{-1}$ for 5 days. When tissues are examined under light microscopy, deterioration of villi morphology, degeneration in brush border structure of epithelial cells, hyperplasia in Goblet cells, and enterocytes due to inflammation, enlargement, and disintegration in lamina propria were detected. Also, hypertrophy of the smooth muscle cells and thickening in the muscularis externa layer were observed. Dysplasia was detected in the small intestine tissues in samples belonging to the highest concentration group.

Keywords: imidacloprid, intestine, histopathology, zebrafish, teleost

İmidakloprid Uygulamasının Zebra Balığı (*Danio rerio*) İnce Bağırsak Dokusunda Oluşturduğu Histopatolojik Etkiler

Özet

Tarımda verimliliği artırmak için pestisit kullanımı gün geçtikçe artmaktadır. Tarımsal alanda kullanılan pestisitler, yağmurlar, yeraltı suları vb. yollarla sucul ekosisteme karışmakta ve sucul canlıların yaşamını tehdit etmektedir. Günümüzde en çok kullanılan pestisit gruplarından biri de neonikotinoidlerdir. Imidakloprid, bu grubun içinde en çok bilinenidir. Bu çalışmada imidaklopridin zebra balığı bağırsak dokusunda yarattığı histopatolojik etkilerin gözlenmesi amaçlanmıştır. Balıklara 5 gün boyunca 9.5, 19 ve 38 $mg.L^{-1}$'lik konsantrasyonlarda imidakloprid uygulanmıştır. Dokular ışık mikroskobunda incelendiğinde, villus morfolojisinde bozulma, epitel hücrelerinde fırçamsı kenar yapısında dejenerasyon, inflamasyona bağlı olarak Goblet hücrelerinde ve enterositlerde hiperplazi, villuslarda birleşme, lamina propriada genişleme ve dağılma, muscularis externa tabakasında kalınlaşma ve bu tabakadaki düz kas hücrelerinde hipertrofi gibi etkiler gözlenmiştir. En yüksek konsantrasyon grubuna ait örneklerde ince bağırsak dokularında displazi tespit edilmiştir.

Anahtar Kelimeler: imidakloprid, ince bağırsak, histopatoloji, zebra balığı, teleost

INTRODUCTION

The use of chemicals in modern agriculture has been increasing efficiency. The most important of these chemicals are undoubtedly pesticides. Pesticides are chemicals used to kill living things like insects, weeds, fungi, bacteria, etc. Pesticides generally have the ability to destroy a wide variety of pests or weeds, but some have been developed against specific pests or pathogens (Jayaraj et al., 2016).

Neonicotinoids are the group of pesticides that are commonly used in agriculture. Pesticides such as imidacloprid, acetamiprid, thiloprid, dinotefuran, nitenpyramide, thiametoxam, and clotianidine are included in the neonicotinoid group (Gupta and Milatovic, 2014).

Imidacloprid [1- (6-Chloro-3-pyridinylmethyl) -N-nitroimidazolidin-2-ylideneamine] is the most widely used and best-known neonicotinoid pesticide in the world (Zhu et al., 2017). It is a broad-spectrum pesticide used for killing pests such as termites, fleas, aphids, fly larvae in many food products, grass and ornamental plant breeding in the agricultural area (Sheets, 2010). Imidacloprid kills insects by acting on the central nervous system. By blocking nicotinic acetylcholine receptors, it

prevents impulse delivery of acetylcholine between nerves, resulting in insect paralysis and eventual death.

As imidacloprid is frequently used in agriculture, they mix with the aquatic ecosystem via storm, runoff, and groundwater (Gupta et al., 2002; Weston et al., 2015). This situation especially affects the life of aquatic organisms. Therefore, in this study, it was aimed to investigate the effects of imidacloprid exposure on zebrafish small intestine tissue by histological methods.

Zebrafish (*Danio rerio*) is a frequently preferred model organism in developmental biology and ecotoxicology studies (Koç et al., 2009; Akbulut et al., 2017). These 4-6 cm silver-colored fish are vertebrate models used to detect toxins in water samples, to reveal diseases and mechanisms caused by environmental toxins. These fish can be used with genetic modifications not only to model human diseases but also to better understand the etiology and mechanisms of environmental diseases and model the effects of environmental toxins on health (Bambino and Chu, 2017).

MATERIALS and METHODS

Test Chemical

Imidacloprid (CAS No: 138261-41-3) was purchased from Sigma Aldrich (Germany).

Test Animal and Experimental Design

Zebrafish were obtained from the Aquaculture Laboratory of the Biology Department of Sakarya University. During the experiment, the fish were kept alive under standard laboratory conditions (28 ± 1ºC temperature, 14 hours light / 10 hours dark photoperiod, 7.0 ± 0.5 pH). The fish used in the experiment were divided into 4 groups (n=10), as 1 control and 3 experimental groups. The imidacloprid concentrations applied to the experimental groups were determined according to the 96-hour LC_{50} concentration (76.08 $mg.L^{-1}$) (Wu et al., 2018). The experimental groups were exposed with 9.5 $mg.L^{-1}$, 19 $mg.L^{-1,}$ and 38 $mg.L^{-1}$ imidacloprid, respectively. After 5 days of exposure, zebrafish were anesthetized with MS-222 and the small intestine tissues were dissected.

Histological Studies

The intestinal tissues were fixed with Bouin's fixative for 24 hours, then the water in the tissue was removed by passing through an ascending series of ethyl alcohol (70%, 95%, 100%). The tissues were cleared in xylene and embedded in paraffin according to the section plane. With the help of microtome, 5 μm thick sections were taken and sections were examined under light microscopy after staining with Hematoxylin-Eosin (H&E) and Periodic Acid Schiff (PAS).

RESULTS

When the samples belonging to the control group were examined, no histopathological changes were observed. Transverse sections taken from the small intestine tissue are examined and the villus structure and layers are photographed. Serosa, muscularis externa, and mucosa layers were observed. Columnar epithelial cells and Goblet cells located individually and scattered between these cells were visualized on the lumen-facing part of the mucosa. Lamina propria was observed just below the epithelial layer. (Figure 1a, 1b).

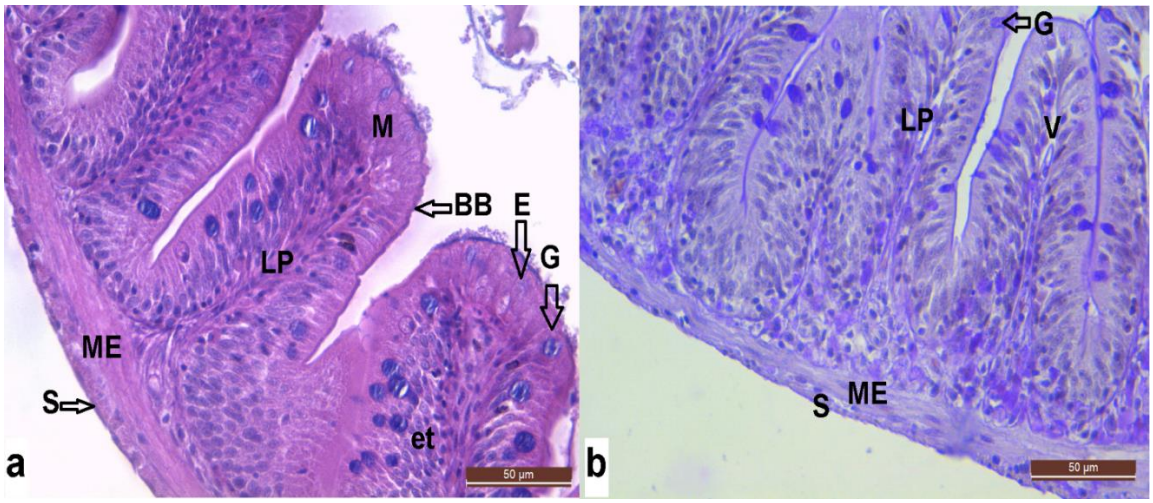

Figure 1. Intestinal tissue at the control group, S: serosa, ME: muscularis externa, LP: lamina propria, M: mucosa, BB: brush border, E: epithelium, G: Goblet cell, V: villus, et: enterocytes, a- H&E stain, b-PAS stain.

In 9.5 mg.L^{-1} imidacloprid exposed group, fusion was detected in the apical of the villi (Figure 2a). Morphological differentiation was observed in some villi structures (Figure 2b, 2d). Compared with the control group, hypertrophy was observed in Goblet cells. In the samples belonging to this group, hyperplasia was detected in the enterocytes within the villi. Vacuolization was observed in lamina propria (Figure 2c). Deformation and disintegration were noticeable in lamina propria (Figure 2d).

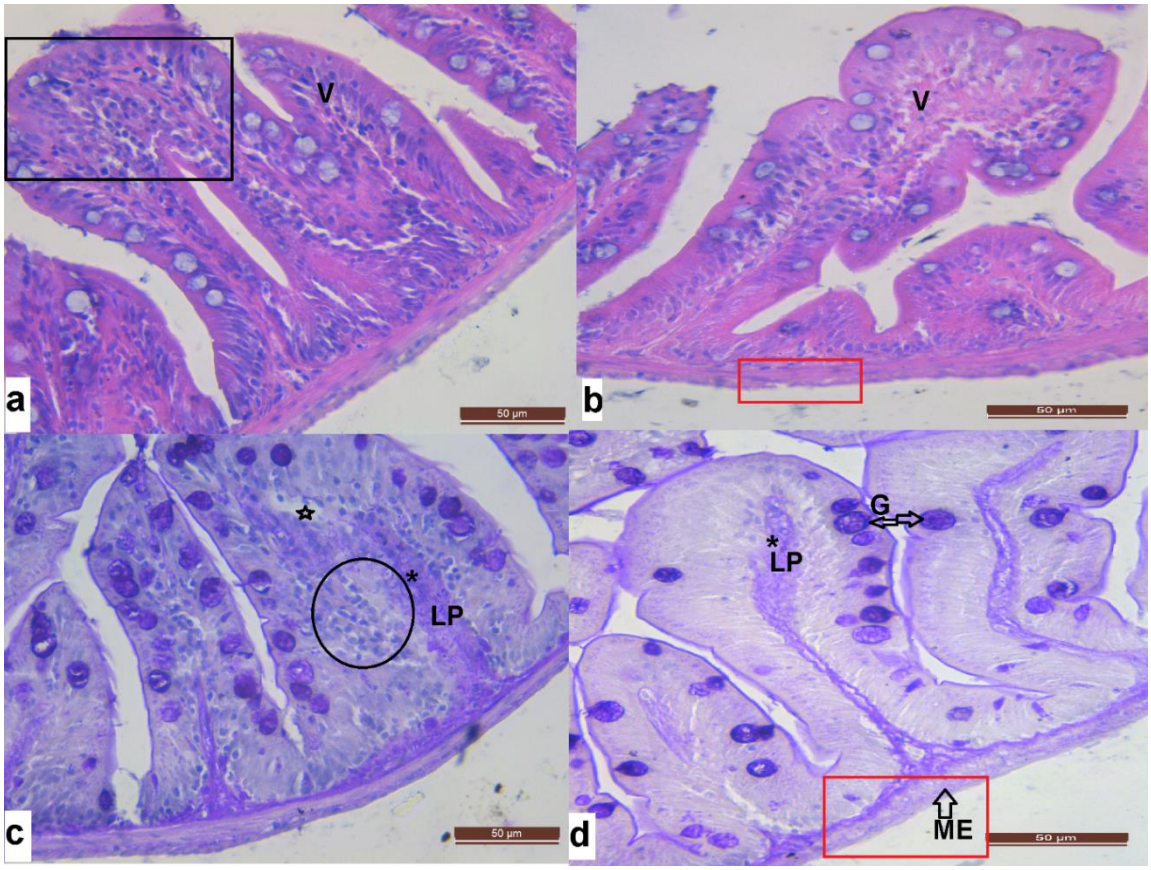

Figure 2. Intestinal tissue of 9.5 mg.L^{-1} imidacloprid exposed group. V: villus, LP: lamina propria, G: Goblet cell, ME: muscularis externa, black rectangle: fusion of villi, red rectangle: disruption of muscularisexterna, star: vacuolization at lamina propria, *: deformation of lamina propria, circle: hyperplasia of enterocytes, a,b- H&E stain, c,d-PAS stain.

In Goblet cells, hyperplasia and loss of cell polarity were observed in 19 mg.L^{-1} imidacloprid exposed group (Figure 3a). When the mucosal region was examined, inflammatory cell infiltrate was seen in lamnina propria (Figure 3a). In the samples belonging to this group, fusion was detected in the villi structures (Figure 3b, 3c, 3d). Thickening of muscularis externa and hypertrophy was observed in smooth muscle cells were also observed. Expansion, disintegration, and bifurcation of connective tissue were noted. (Figure 3c).

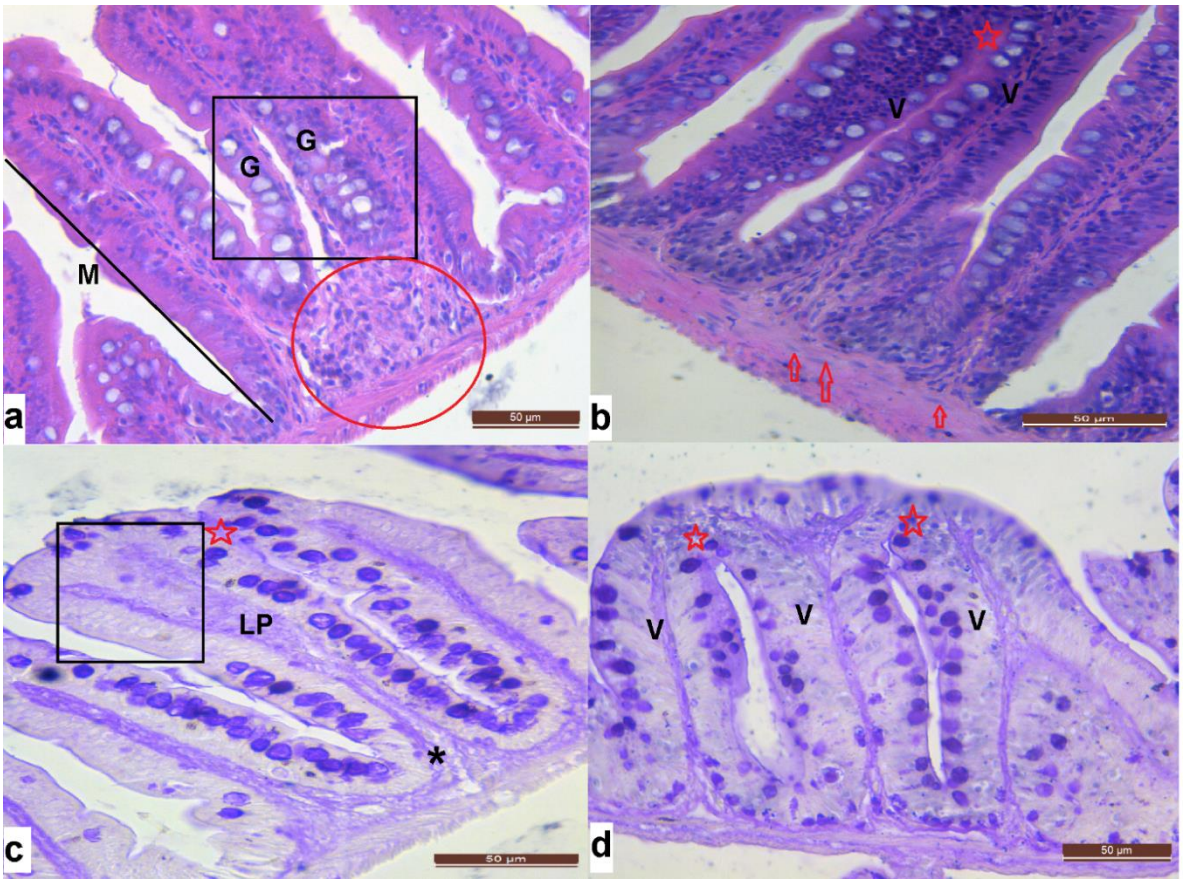

Figure 3. Intestinal tissue of 19 mg.L^{-1} imidacloprid exposed group. V: villus, M: mucosa, G: Goblet cell, LP: lamina propria, rectangle: loss of cell polarity at Goblet cells, circle: inflammatory cell infiltrate, square: branching of lamina propria, arrow: hipertrophy of smooth muscle cells, star: fusion of villi, a,b- H&E stain, c,d-PAS stain.

When the tissues in the 38 mg.L^{-1} imidacloprid exposed group were compared with other groups, the histopathological effects were found more severe. In this group, total fusion, increase in the number of enterocytes, inflammatory cell infiltrate were observed in intestinal tissues (Figure 4a). Disruption in villus morphology and degeneration in lamina propria were noteworthy (Figure 4b, 4c, 4d). Bifurcation (Figure 4b, 4d) and enlargement (Figure 4c) were detected in the lamina propria. The brush border structure of epithelial cells was disrupted (Figure 4b). The formation of pseudocrypt in villi was seen (Figure 4c). In muscularis externa, vacuolization (figure 4a, 4b, 4c, 4d) and thickening (Figure 4c) were displayed. It was observed that some smooth muscle cells in the muscularis externa were extensive hypertrophic and others were necrotic (Figure 4c). As seen in the 19 mg.L^{-1} exposed group, loss of cell polarity and hypertrophy were noticed in Goblet cells (Figure 4a, 4d). Nuclear atypia, cellular pleomorphism and aberrant mitotic cells, which are indicators of dysplasia were visualized in the cells of the villi (Figure 4d).

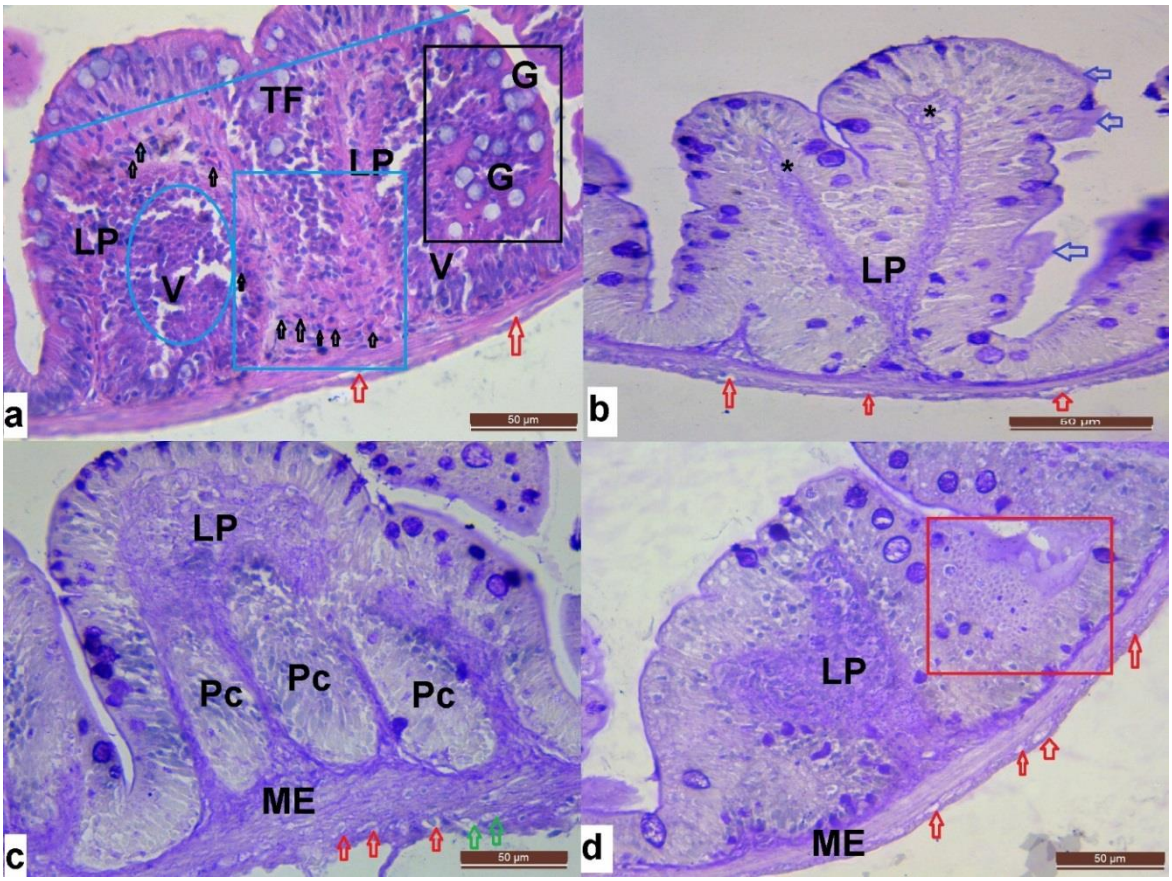

Figure 4. Intestinal tissue of 38 mg.L^{-1} imidacloprid exposed group. V: villus, G: Goblet cell, LP: lamina propria, ME: muscularis externa, TF: total fusion, Pc: pseudocrypt, black rectangle: loss of cell polarity at Goblet cells, blue circle: hyperplasia of enteroytes, blue rectangle: inflammatory cell infiltrate, black arrow: erytrocytes, blue arrow: disruption of brush border, red arrow: hypertorphy of smooth muscle cells, red rectangle: nuclear atypia and aberrant mitotic cells, a,b- H&E stain, c,d-PAS stain.

DISCUSSION and CONCLUSION

Pesticide residues can be an important source of contamination for environmental factors such as air, water, and soil. This situation can become a constant threat to the lives of plant and animal communities (Jayaraj et al., 2016).

Studies examining the damage caused by environmental pollutants in the intestinal tissue of fish by histological methods are limited. Velmurugan et al. (2007) investigated the histopathological effects of lambda-cyhalothrin, a pyrethroid pesticide, in intestinal tissues of *Cirrhinus mrigala*. They detected atrophy in epithelial cells and eosinophil infiltration in lamina propria in intestinal tissues treated with lambda-cyhalothrin.

The histopathological effects of lindane, one of the organochlorine pesticides, on the intestine tissue of *Channa punctata*, a teleost fish, were investigated (Bhattacharjee and Das, 2015). As a result of the study, it was concluded that lindane exposure caused inflammatory cell infiltration in intestinal tissues, vacuolization in epithelial cells, proliferation in Goblet cells, and induced necrosis. In a like manner, in our study, inflammatory cell infiltration and Goblet cell count increased. Similarly, mancozeb, an organophosphate pesticide, causes histopathological changes in the intestine tissue of fish like other pesticides. Das and Gupta (2013) examined the effects of mancozeb on *Esomus danricus* in the intestinal tissues using histological methods and as a result of the study, they observed effects such as ulceration and vacuolization and chronic inflammatory cell infiltration in the mucosa layer. Unlike this study, no ulceration was found in our study.

The histological effects of thiodan (endosulfan), one of the pesticides frequently used in agriculture, were investigated on mosquitofish, *Gambusia affinis* intestine tissues by Cengiz et al. (2001). Lymphocyte accumulation, edema, degeneration, disintegration of villi, picnotic nuclei, and necrosis were detected in lamina propria. Unlike this study, edema in the lamina propria and

fragmentation of the villi were not observed in our study. Similar to the findings of Cengiz et al. (2001), cells with edema, picnotic nuclei, and atypical nuclei were also observed.

In another study examining the histopathological changes of 2,4-dichlorophenoxyacetic acid in zebrafish intestine tissue, findings such as hyperplasia in goblet cells, degeneration and edema formation in villi structures, and necrosis and atrophy in epithelial cells were observed (Yön Ertuğ et al., 2014). The results of this study are consistent with our findings.

Zebrafish intestinal morphology is quite homologous to that of higher vertebrates (Brugman, 2016). Therefore, the results obtained here will also be important in terms of understanding the damage caused by environmental pollutants in higher vertebrates.

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